Figure 1: Various synthetic pathways for the biosynthesis of DHA (docosahexaenoic acid)

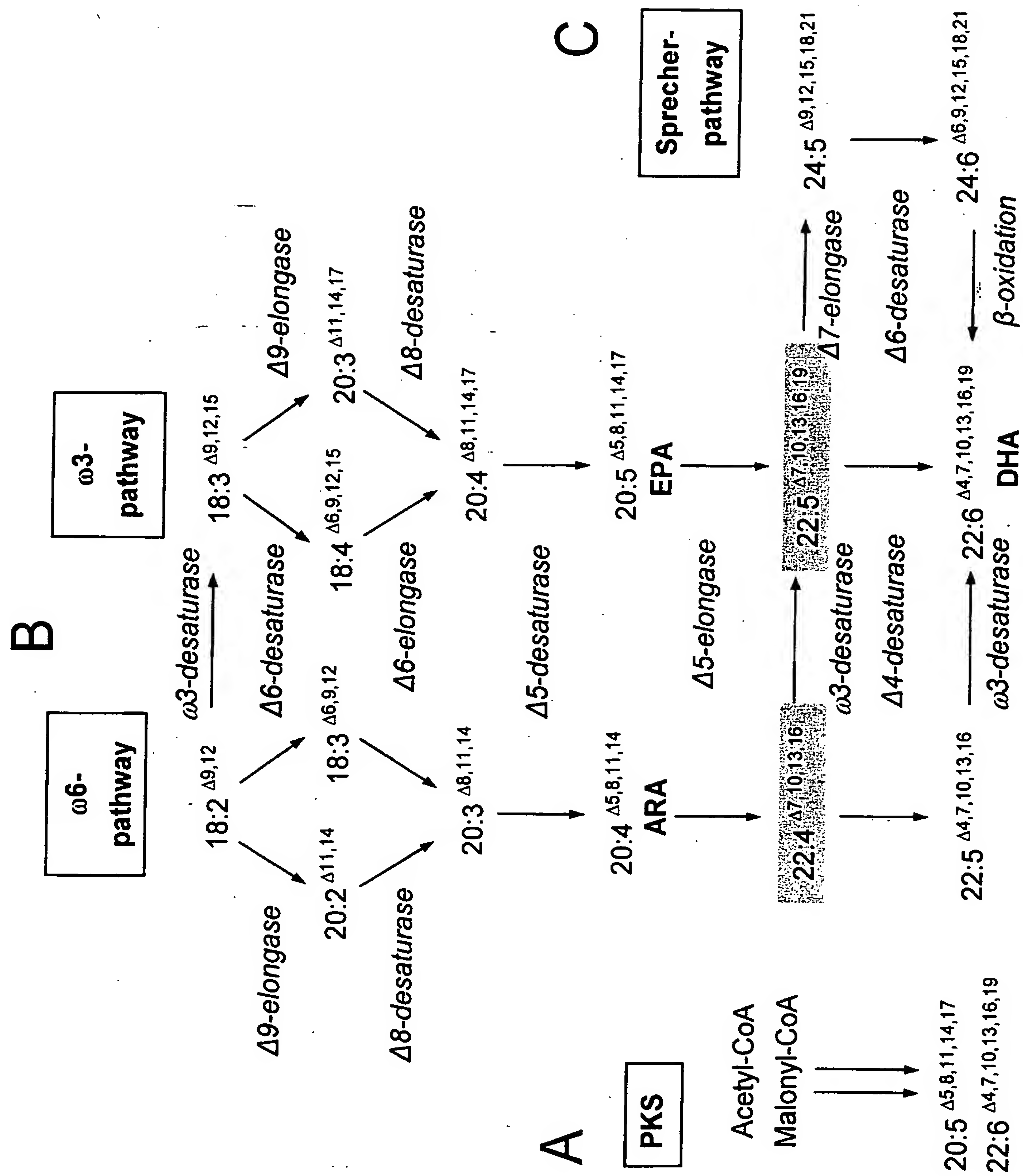


Figure 2: Substrate specificity of the Δ5-elongase (SEQ ID NO: 53) for various fatty acids

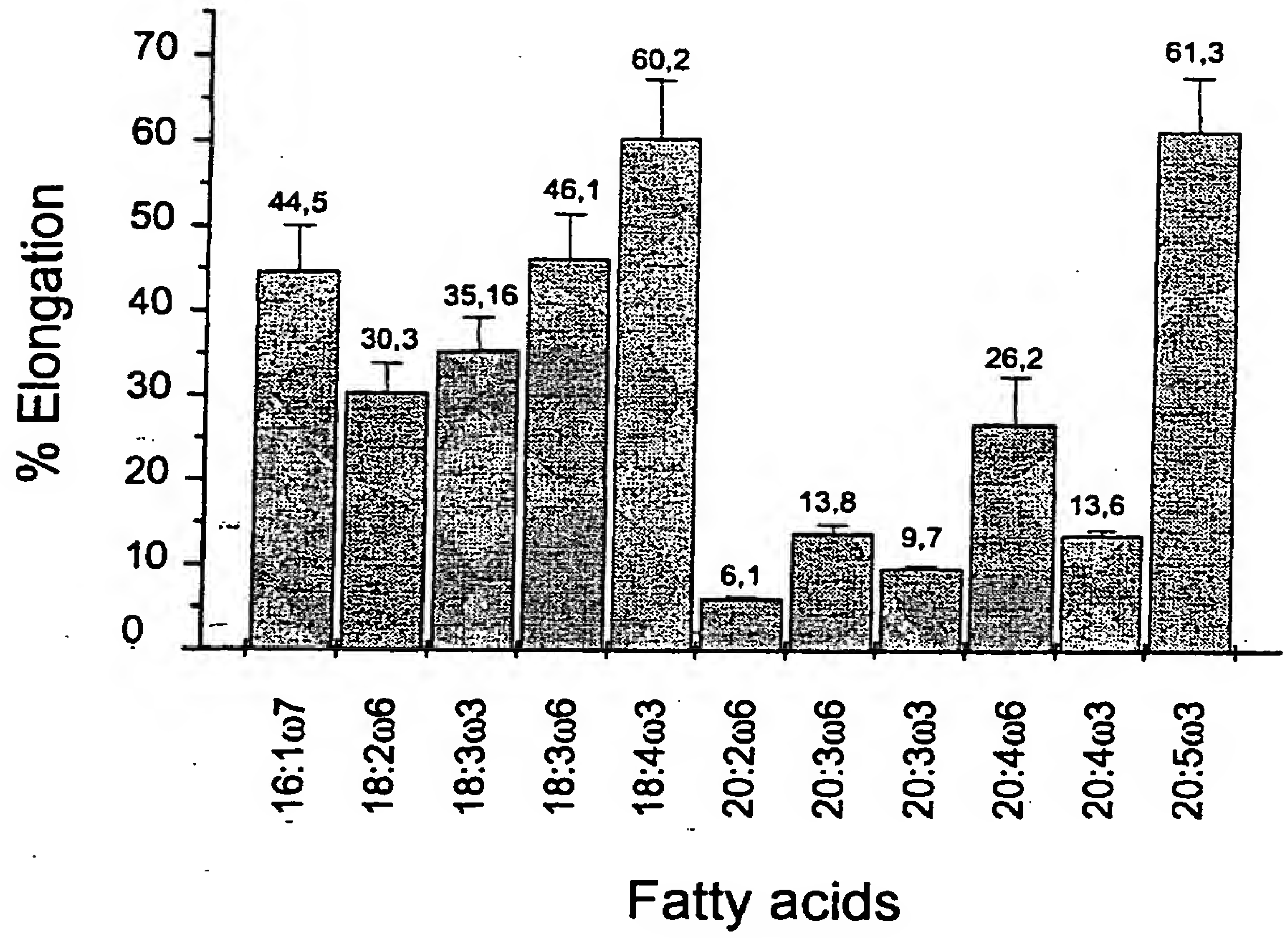


Figure 3: Reconstitution of DHA biosynthesis in yeast starting from 20:5ω3.

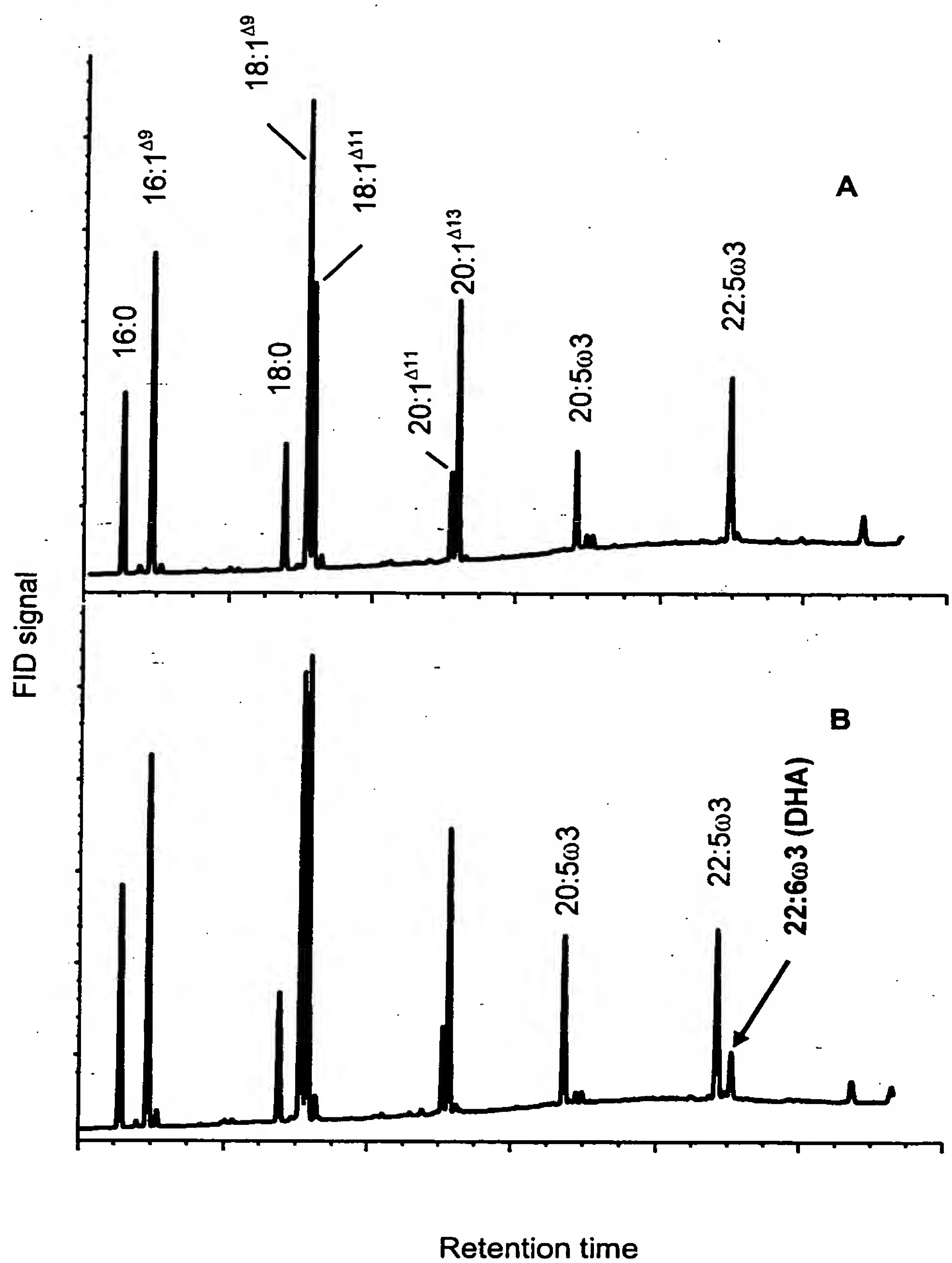


Figure 4: Reconstitution of DHA biosynthesis in yeast starting from 18:4ω3.

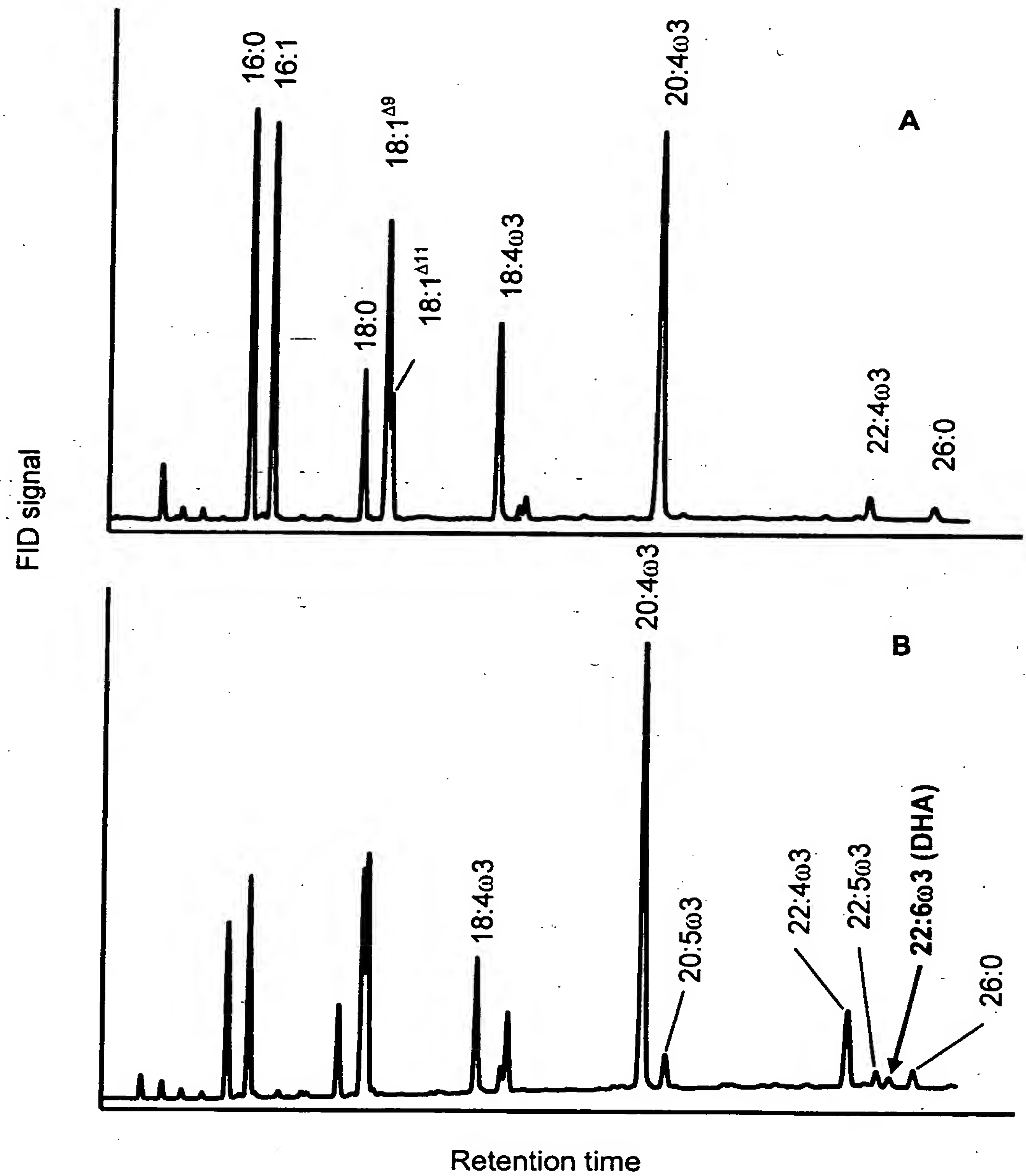


Figure 5: Fatty acid composition (in mol%) transgenic yeasts which had been transformed with the vectors pYes3-OmELO3/pYes2-EgD4 or pYes3-OmELO3/pYes2-EgD4+pESCLeu-PtD5. The yeast cells were grown in minimal medium with tryptophan and uracil / and leucin in the presence of 250 µM 20:5$^{\Delta 5,8,11,14,17}$ and 18:4$^{\Delta 6,9,12,15}$, respectively. The fatty acid methyl esters were obtained from cell sediments by acid methanolysis and analyzed via GLC. Each value represents the mean value (n=4) ± standard deviation.

| | pYes3-OmELO/pYes2-EgD4 | pYes3-OmELO/pYes2-EgD4 EgD4 + pESCLeu-PtD5 |
|---|---|---|
| Fatty acids | Feeding with 20:5$^{\Delta 5,8,11,14,17}$ | Feeding with 18:4$^{\Delta 6,9,12,15}$ |
| 16:0 | 9.35 ± 1.61 | 7.35 ± 1.37 |
| 16:1 $^{\Delta 9}$ | 14.70 ± 2.72 | 10.02 ± 1.81 |
| 18:0 | 5.11 ± 1.09 | 4.27 ± 1.21 |
| 18:1 $^{\Delta 9}$ | 19.49 ± 3.01 | 10.81 ± 1.95 |
| **18:1 $^{\Delta 11}$** | **18.93 ± 2.71** | **11.61 ± 1.48** |
| **18:4 $^{\Delta 6,9,12,15}$** | - | **7.79 ± 1.29** |
| **20:1 $^{\Delta 11}$** | **3.24 ± 0.41** | **1.56 ± 0.23** |
| **20:1 $^{\Delta 13}$** | **11.13± 2.07** | **4.40 ± 0.78** |
| **20:4$^{\Delta 8,11,14,17}$** | - | **30.05 ± 3.16** |
| **20:5$^{\Delta 5,8,11,14,17}$** | **6.91± 1.10** | **3.72 ± 0.59** |
| **22:4$^{\Delta 10,13,16,17}$** | - | **5.71 ± 1.30** |
| **22:5 $^{\Delta 7,10,13,16,19}$** | **8.77 ± 1.32** | **1.10 ± 0.27** |
| **22:6 $^{\Delta 4,7,10,13,16,19}$** | **2.73 ± 0.39** | **0.58 ± 0.10** |

Figure 6: Feeding experiment for determining the functionality and substrate specificity with yeast strains

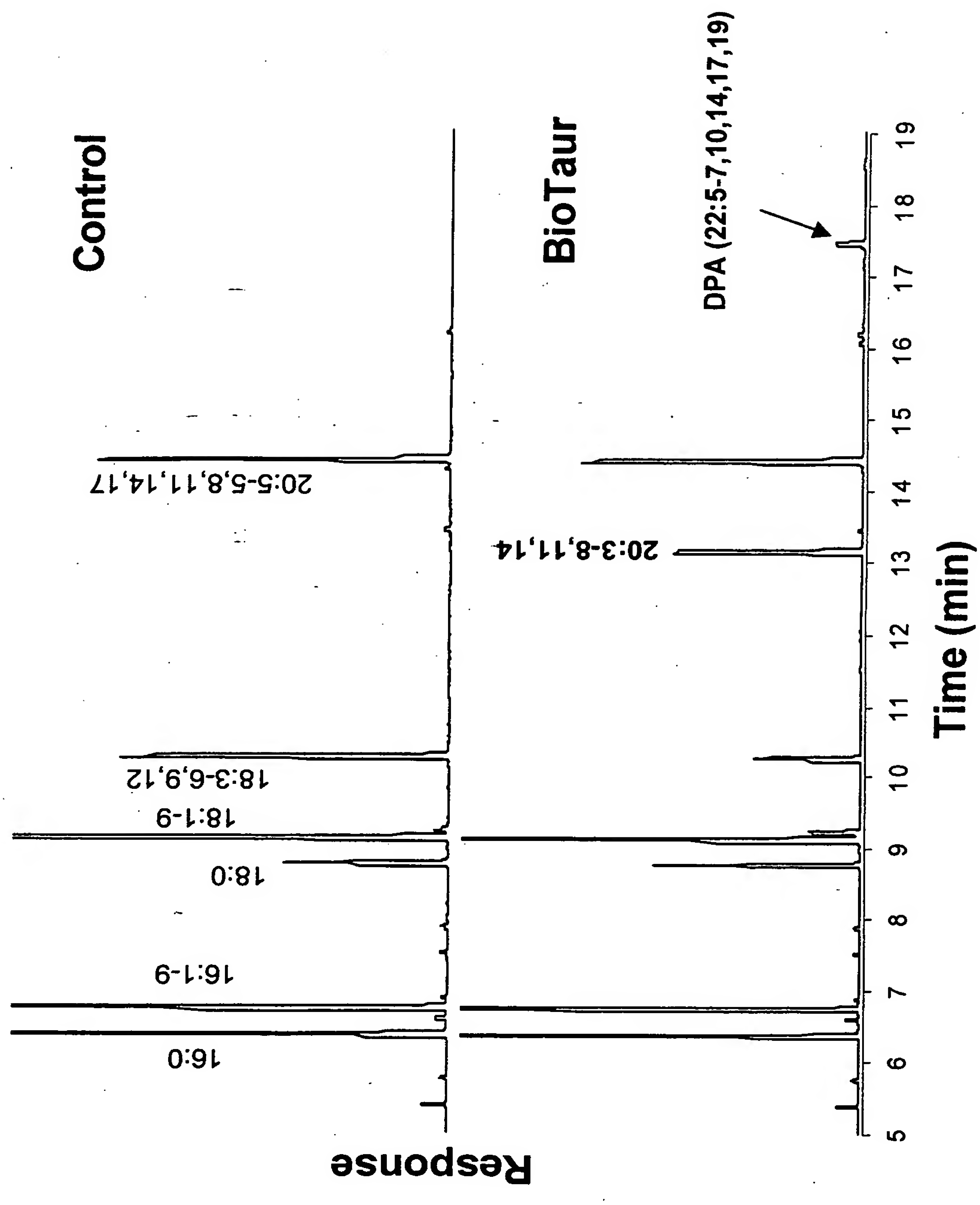
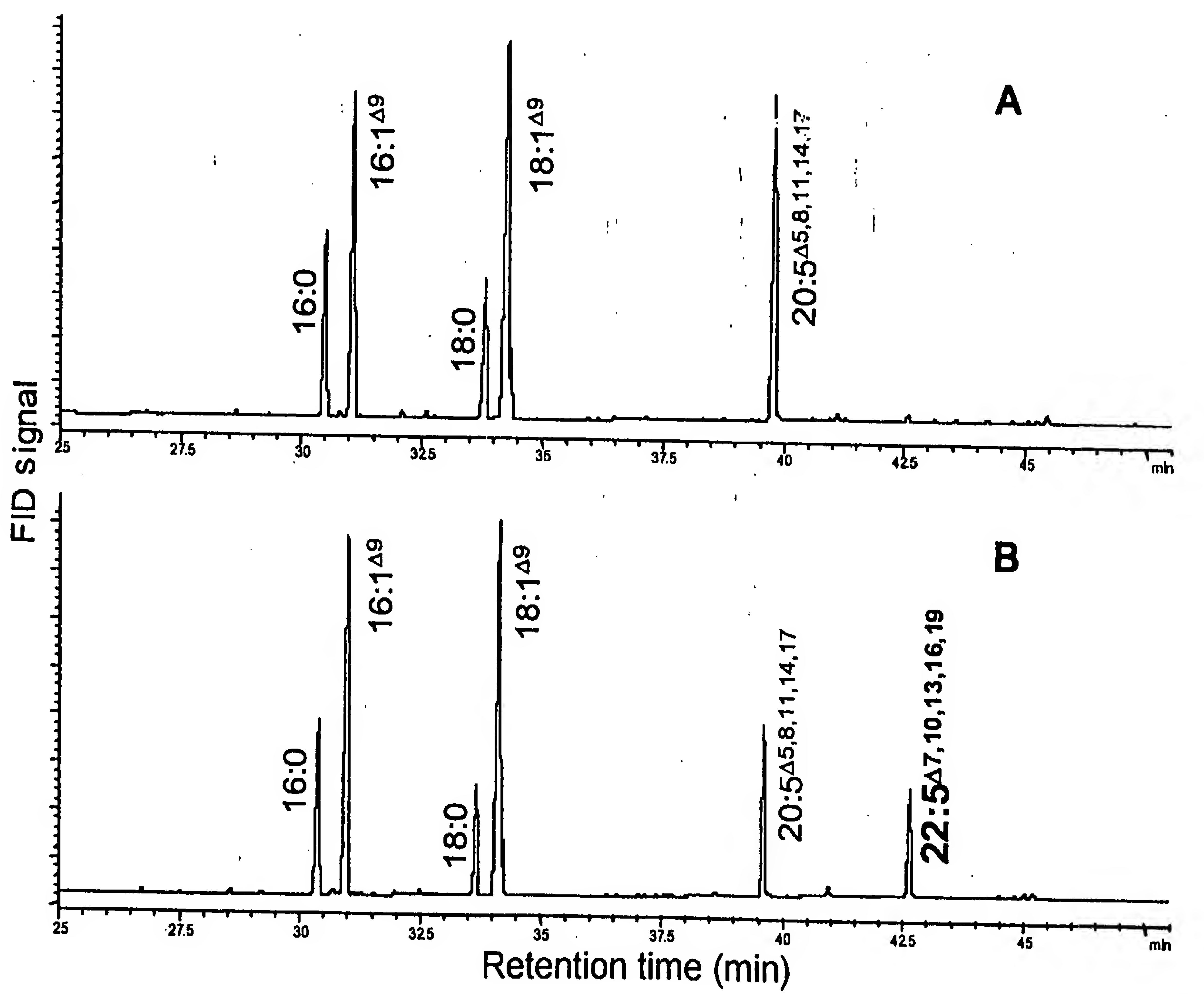


Figure 7: Elongation of eicosapentaenoic acid by OtElo1



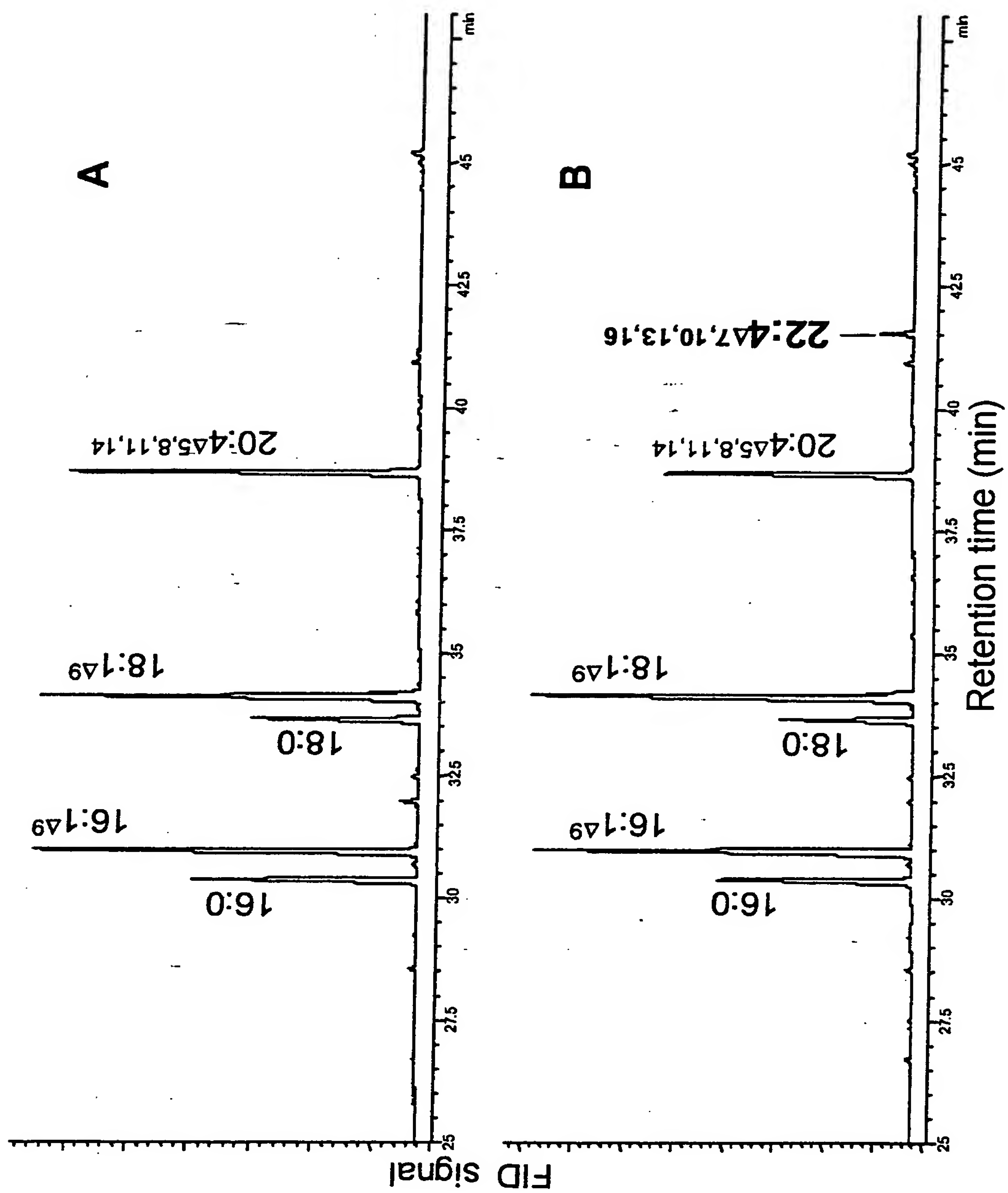



Figure 8: Elongation of arachidonic acid by OtElo1

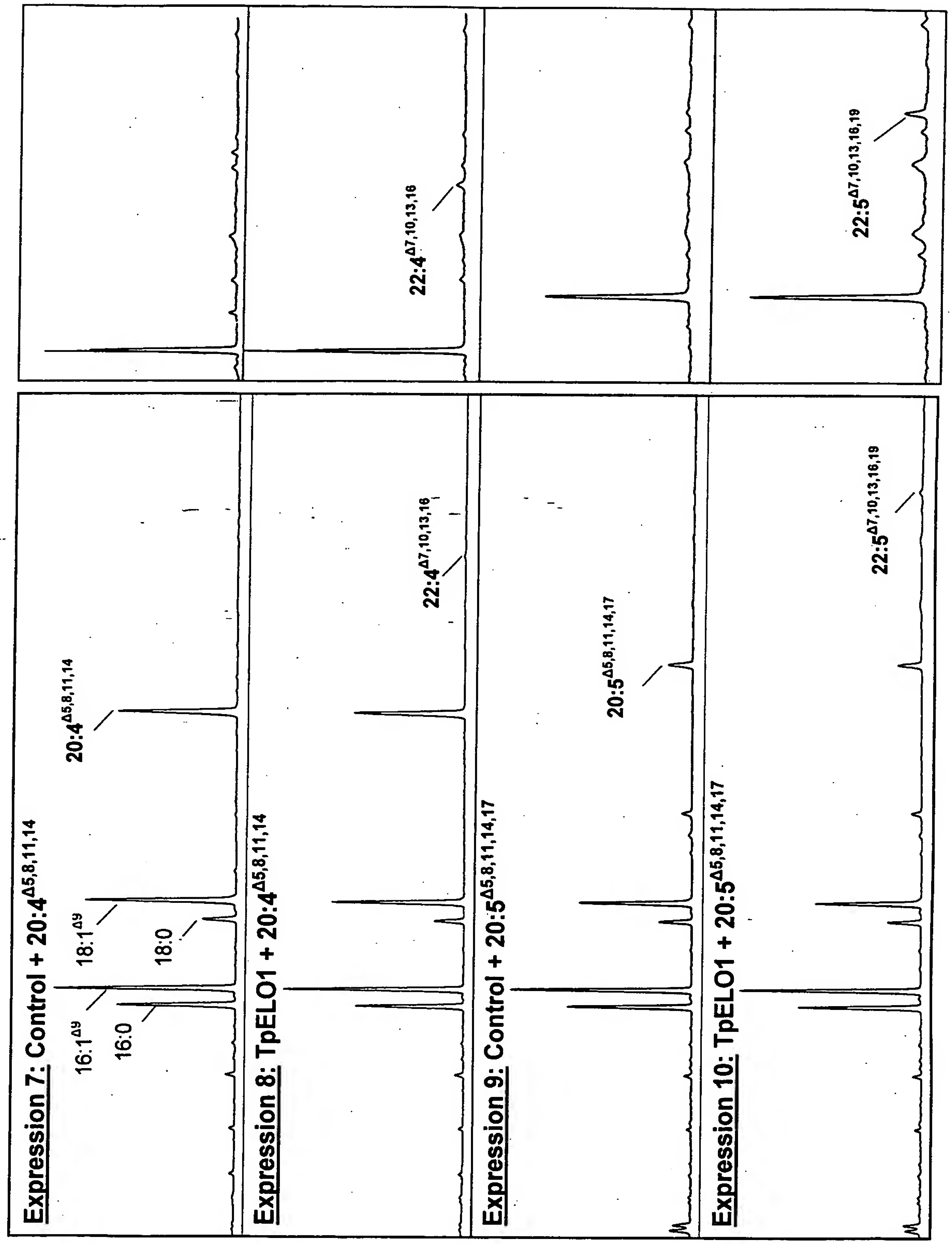



Figure 9: Expression of TpELO1 in yeast

Figure 10: Expression of TpELO3 in yeast

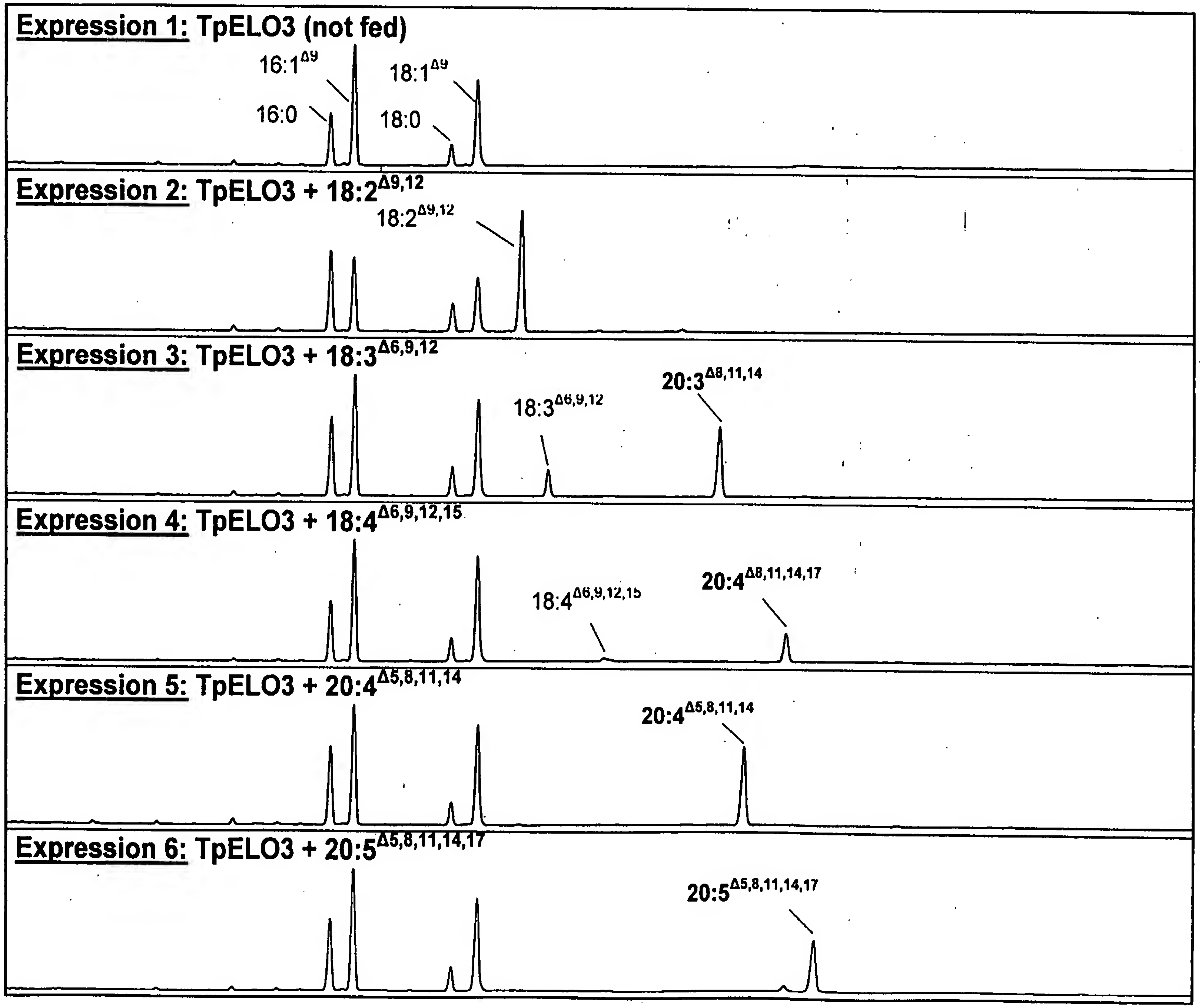


Figure 11: Expression of Thraustochytrium Δ5-elongase TL16/pYES2.1 in yeast

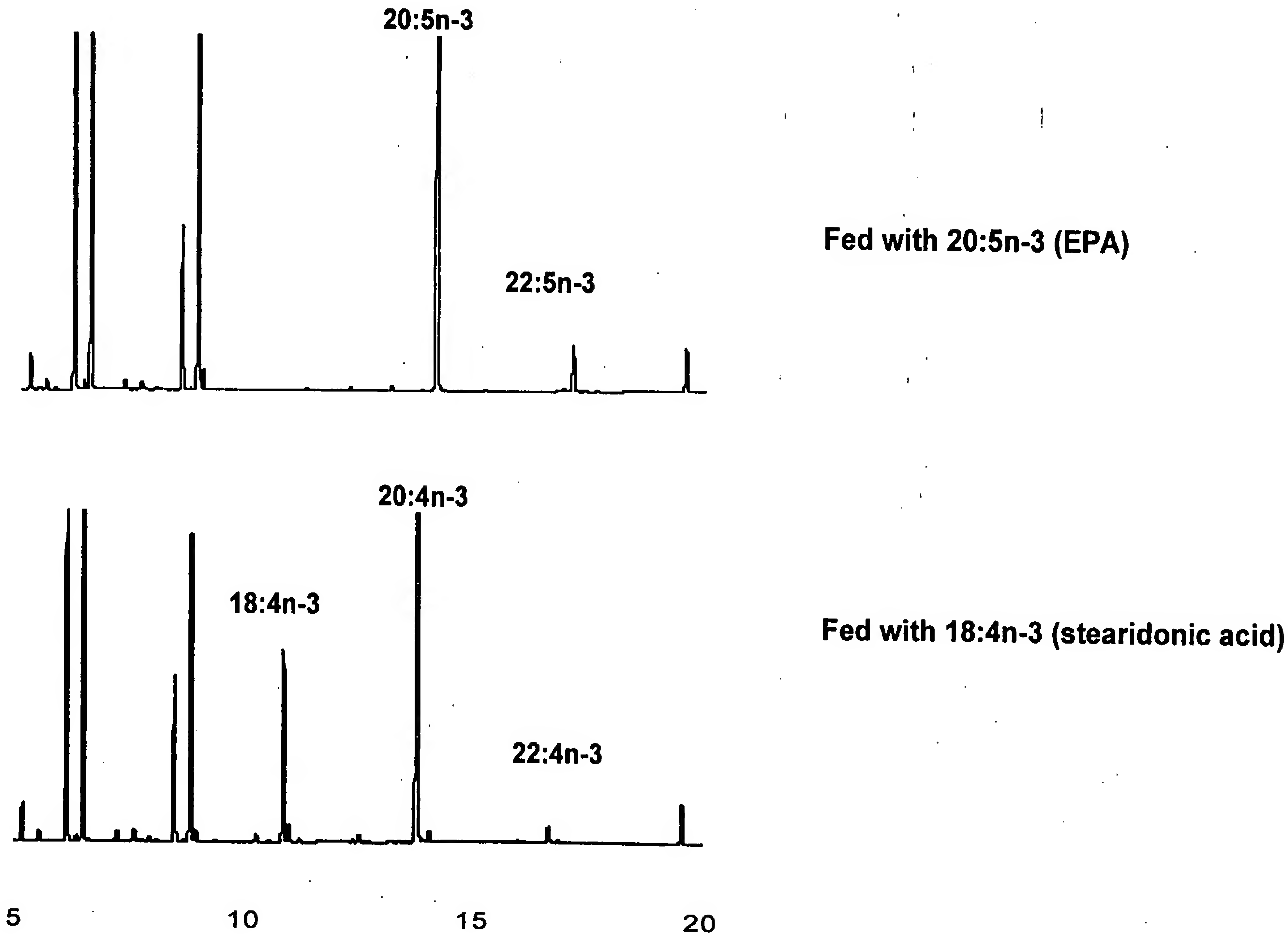


Figure 12: Desaturation of linoleic acid (18:2 ω6-fatty acid) to give α-linolenic acid (18:3 ω3-fatty acid) by Pi-omega3Des.

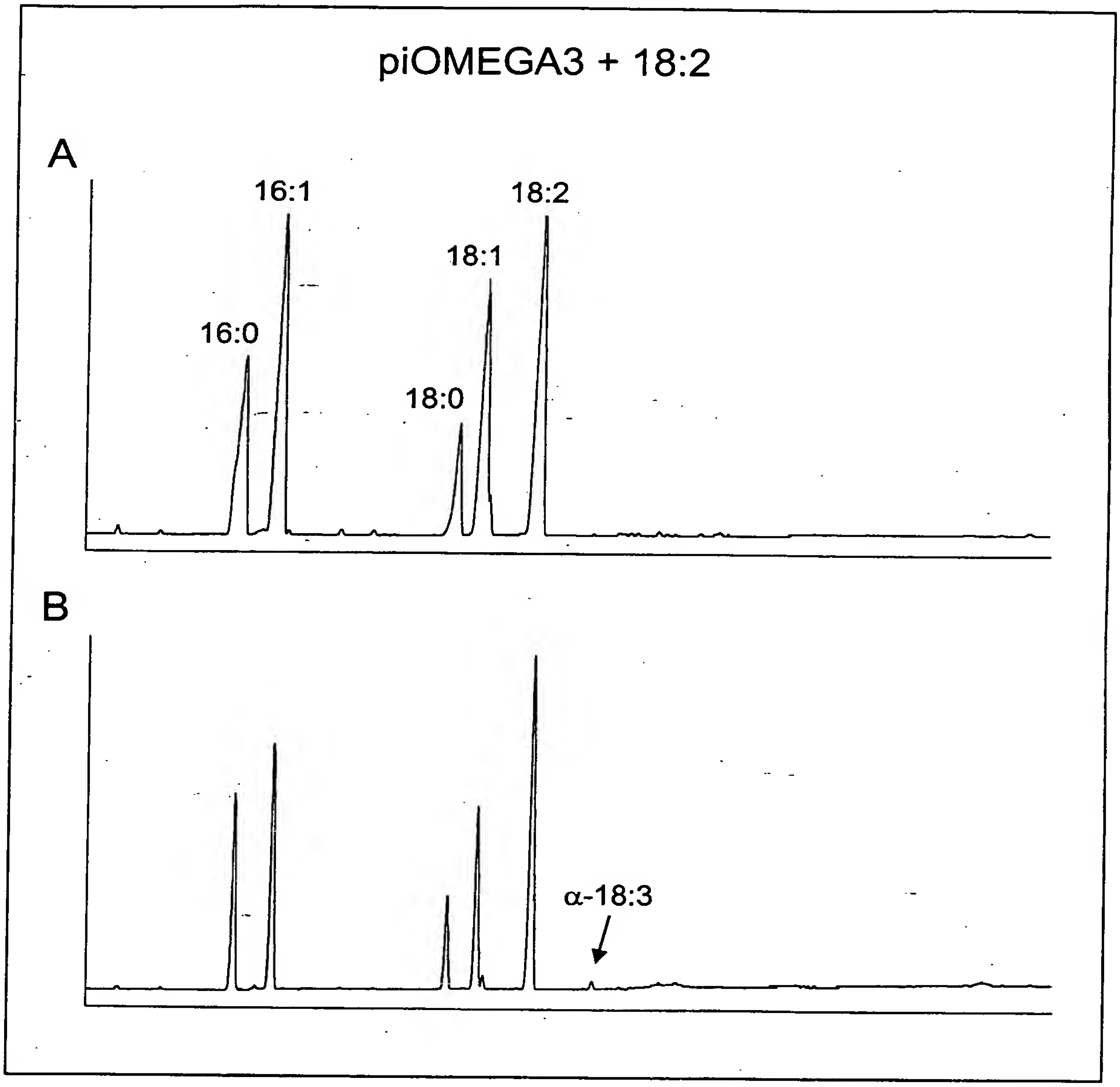


Figure 13: Desaturation of γ-linolenic acid (18:3 ω6-fatty acid) to give stearidonic acid (18:4 ω3 fatty acid) by Pi-omega3Des.

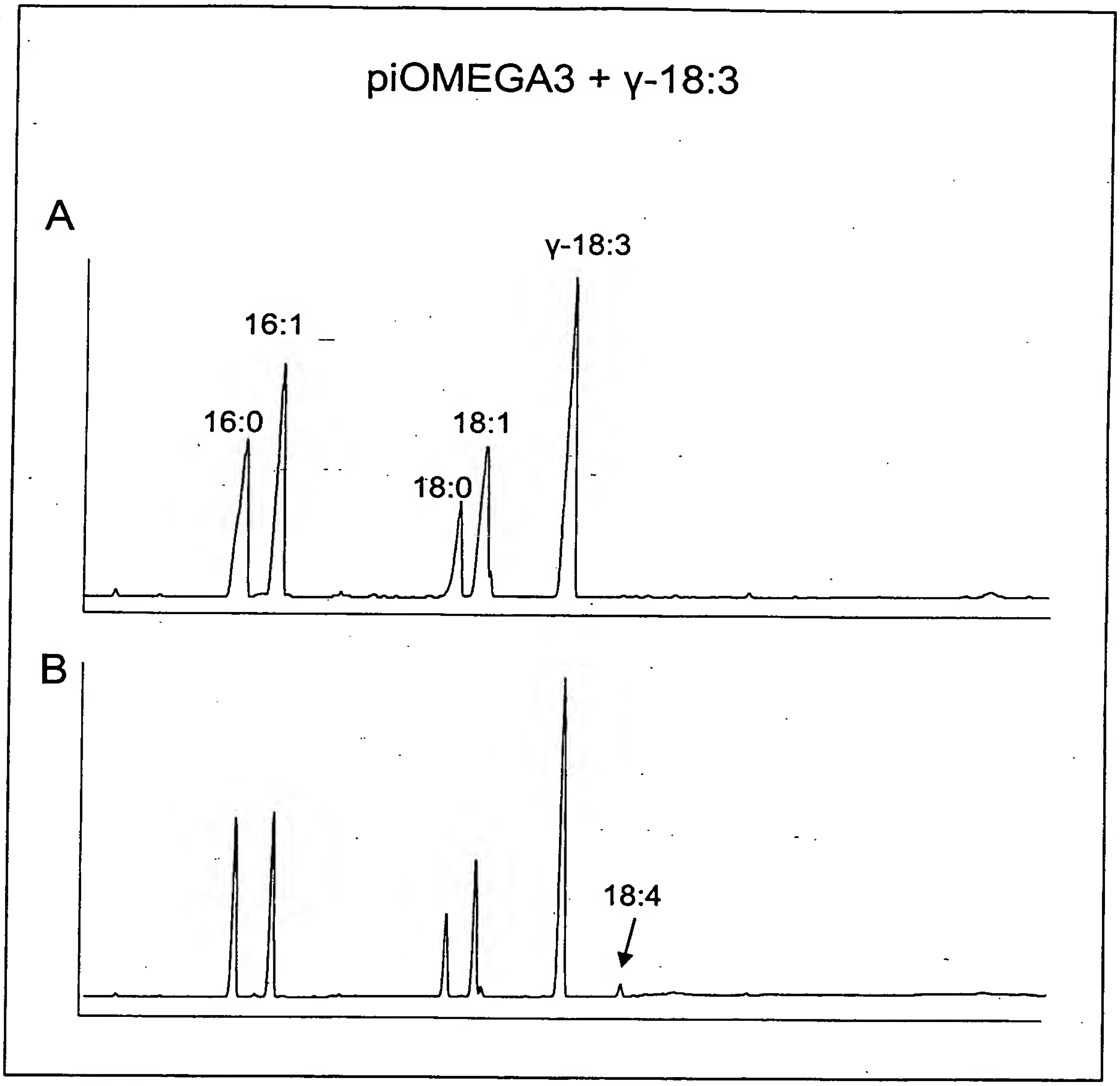


Figure 14: Desaturation of C20:2 ω6-fatty acid to give C20:3 ω3-fatty acid by Pi-omega3Des.

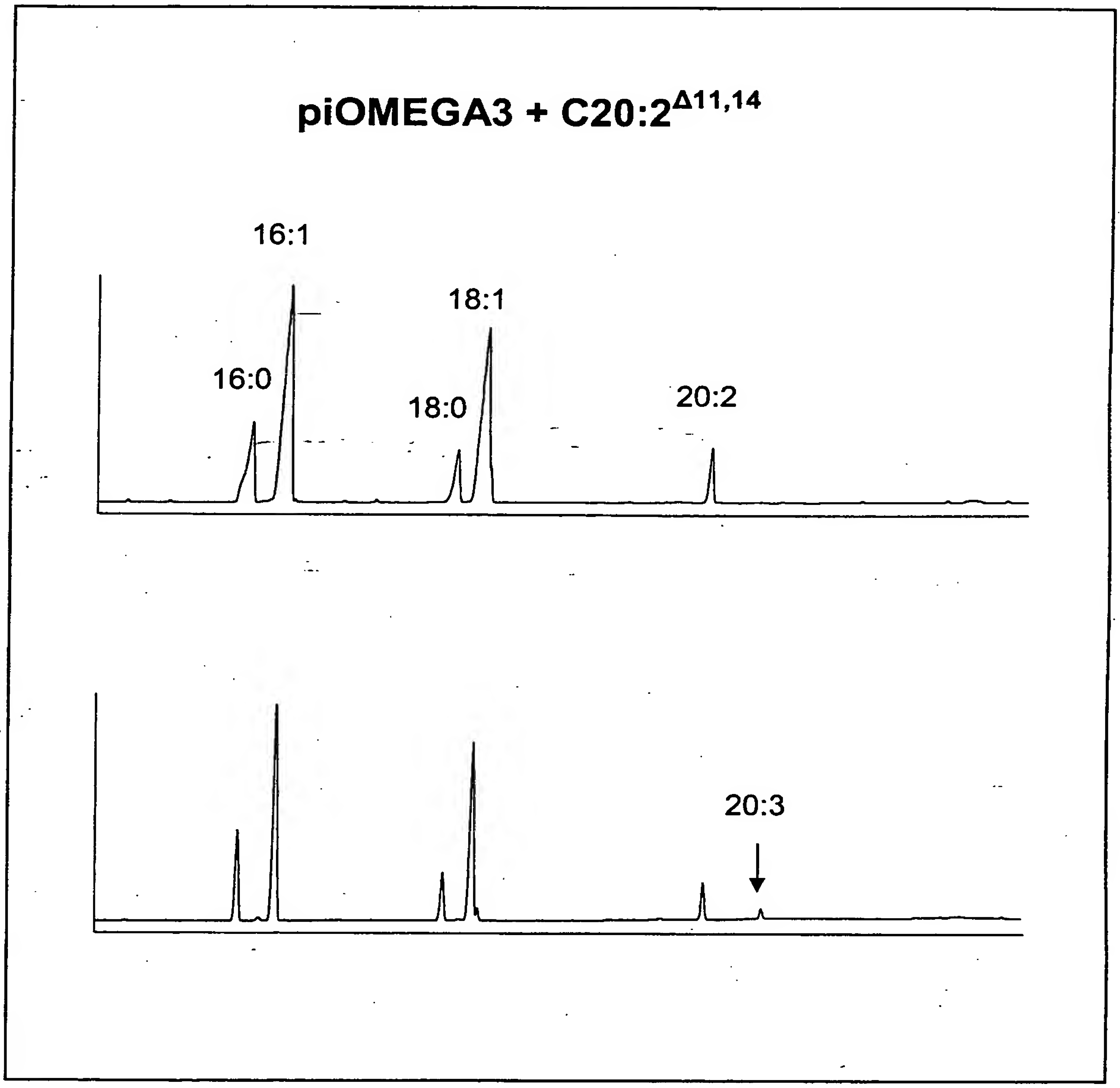


Figure15: Desaturation of C20:3 ω6-fatty acid to give C20:4 ω3-fatty acid by Pi-omega3Des.

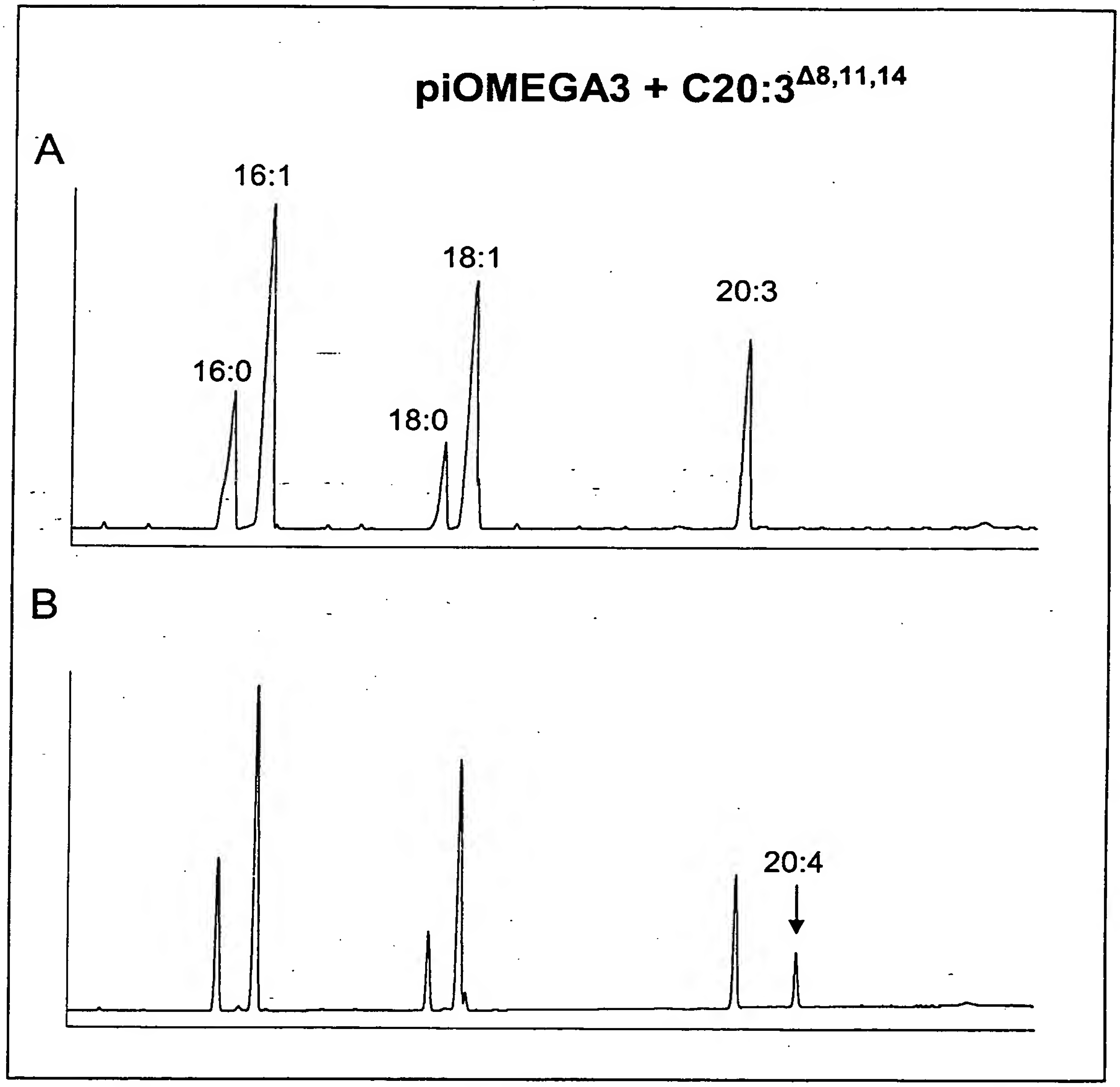


Figure16: Desaturation of arachidonic acid (C20:4 ω6-fatty acid) to give eicosapentaenoic acid (C20:5 ω3-fatty acid) by Pi-omega3Des.

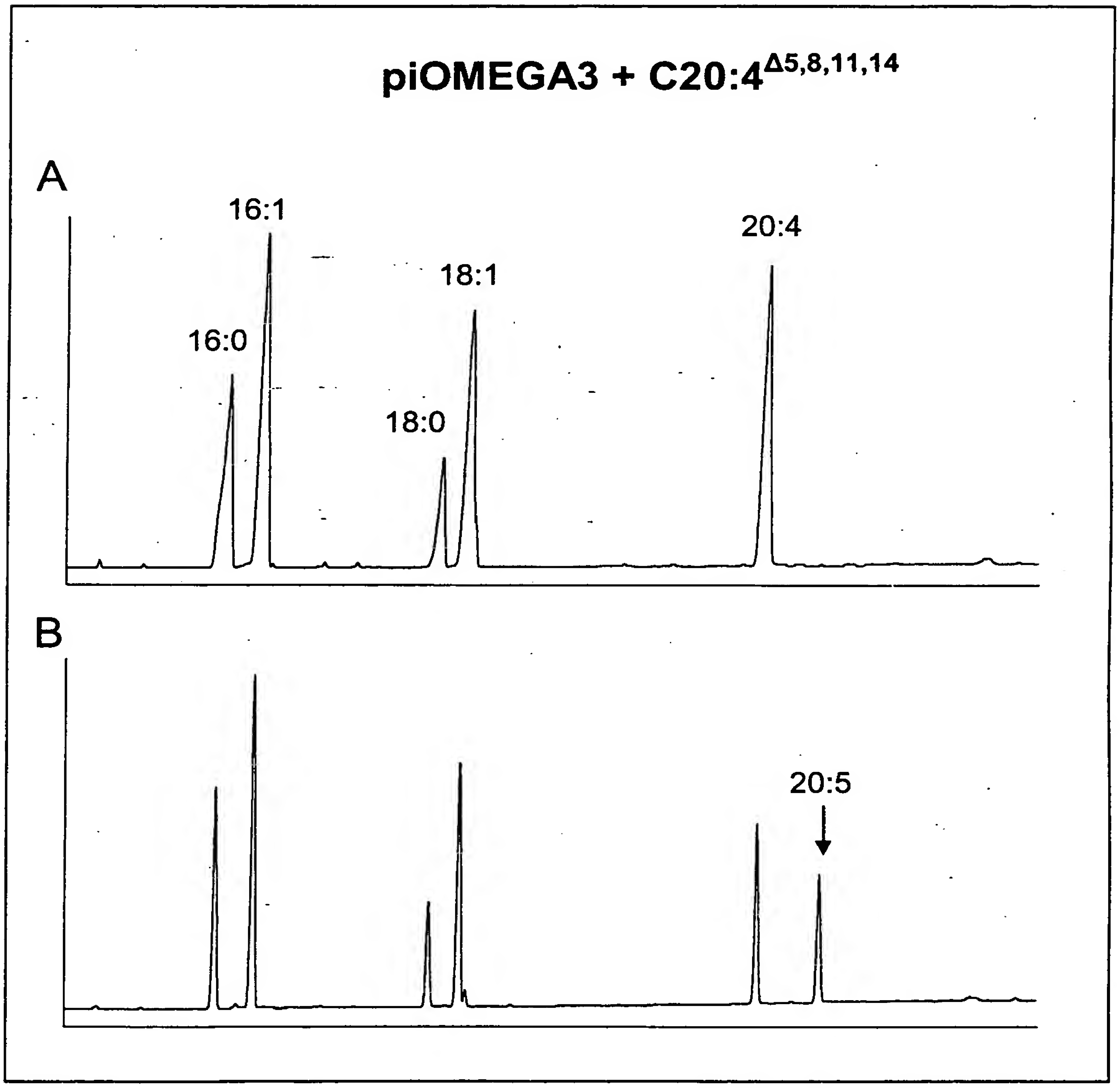


Figure 17: Desaturation of docosatetraenoic acid (C22:4 ω6-fatty acid) to give docosapentaenoic acid (C22:5 ω3-fatty acid) by Pi-omega3Des.

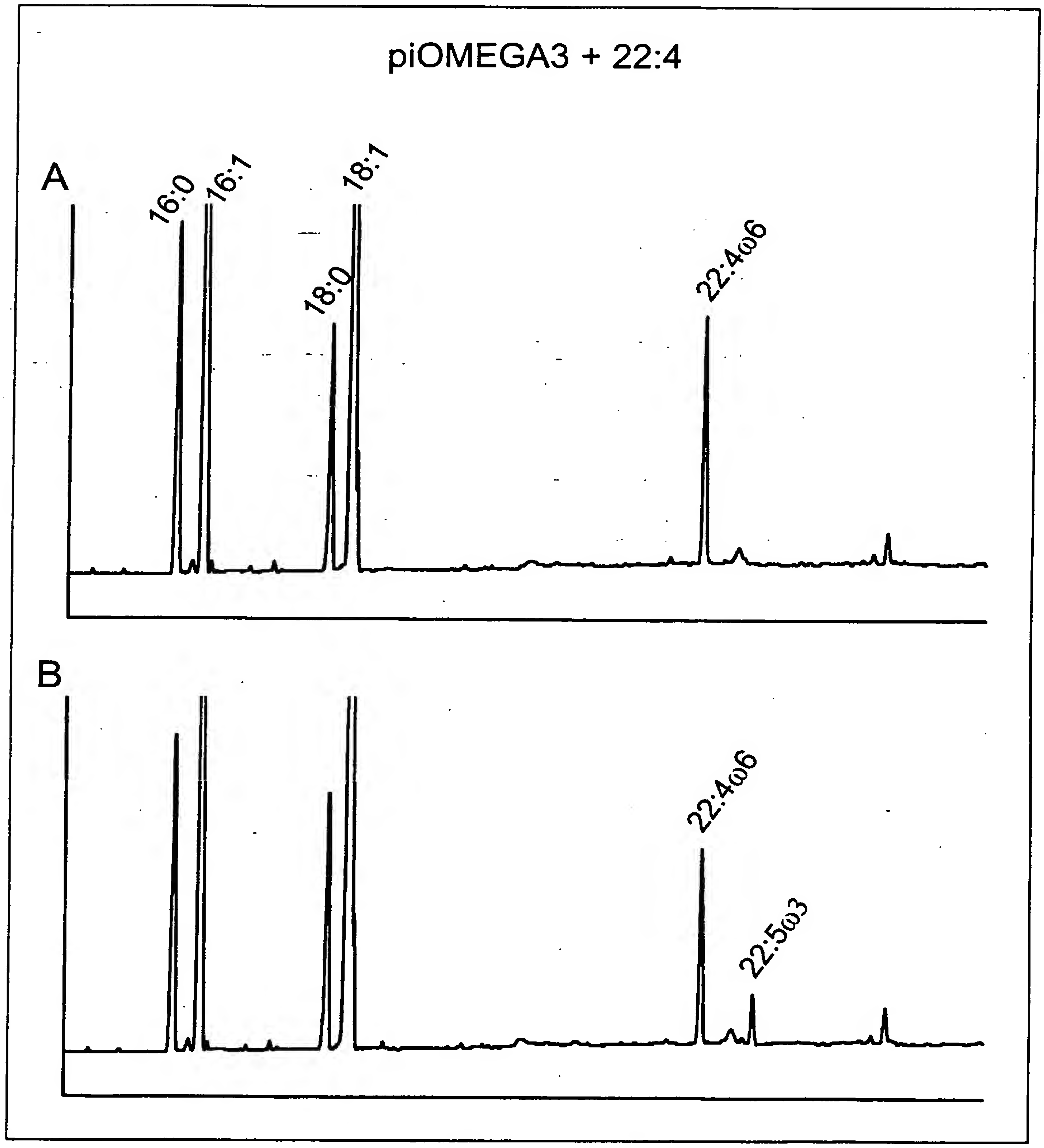
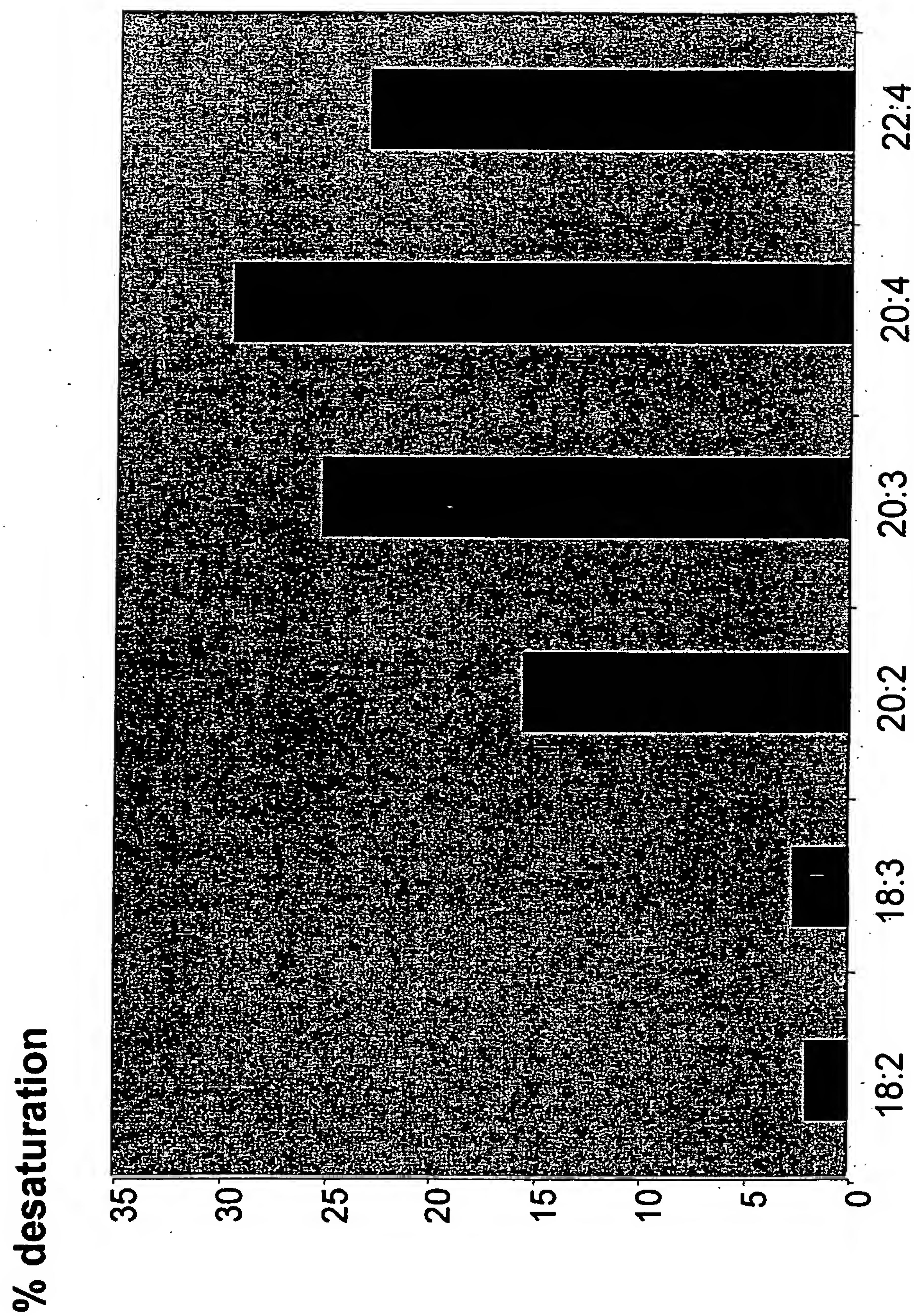


Figure 18: Substrate specificity of Pi-omega3Des for various fatty acids



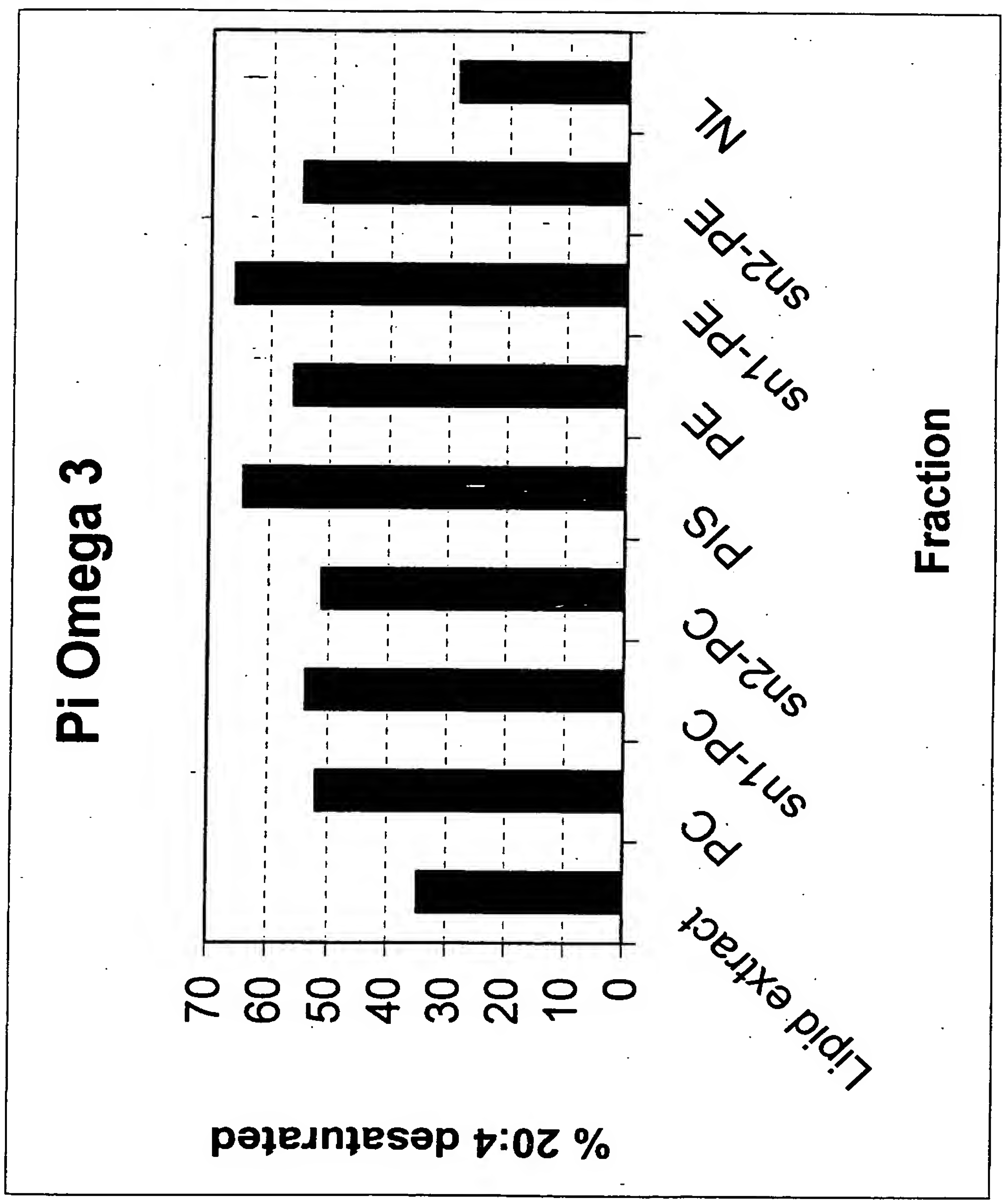



Figure 19: Desaturaturation of phospholipid-bound arachidonic acid to EPA by Pi-Omega3Des

Figure 20: Conversion by OtDes6.1 of linoleic acid (arrow) into γ-linolenic acid (γ-18:3).

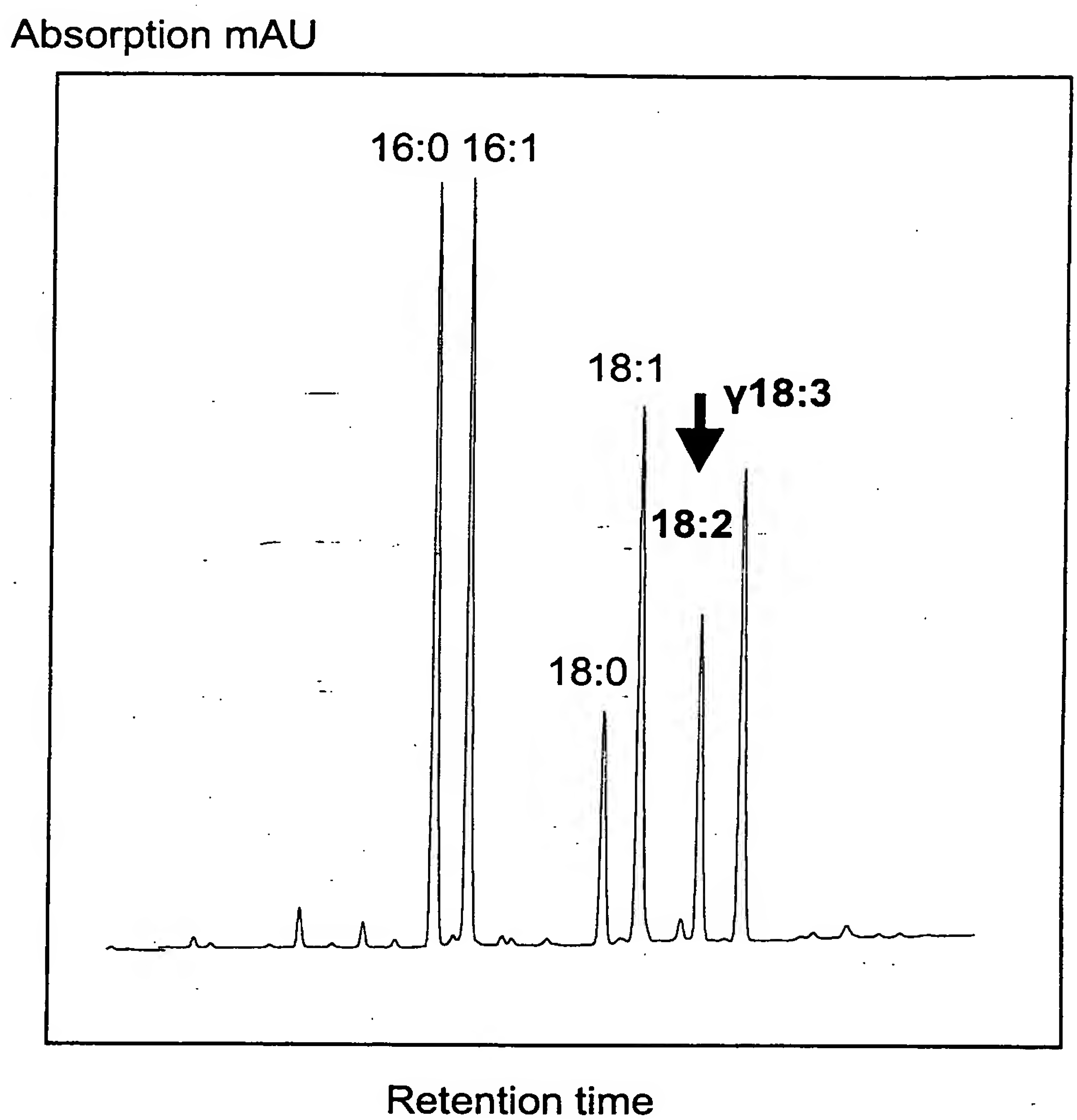


Figure 21: Conversion of linoleic acid and Umsetzung von Linolsäure und α-lLinolenic acidsäure (A aund C), and sowie rReconstitution of the ARA and EPA synthetic pathways, respectively, in yeast (B and D) in the presence of OtD6.1.

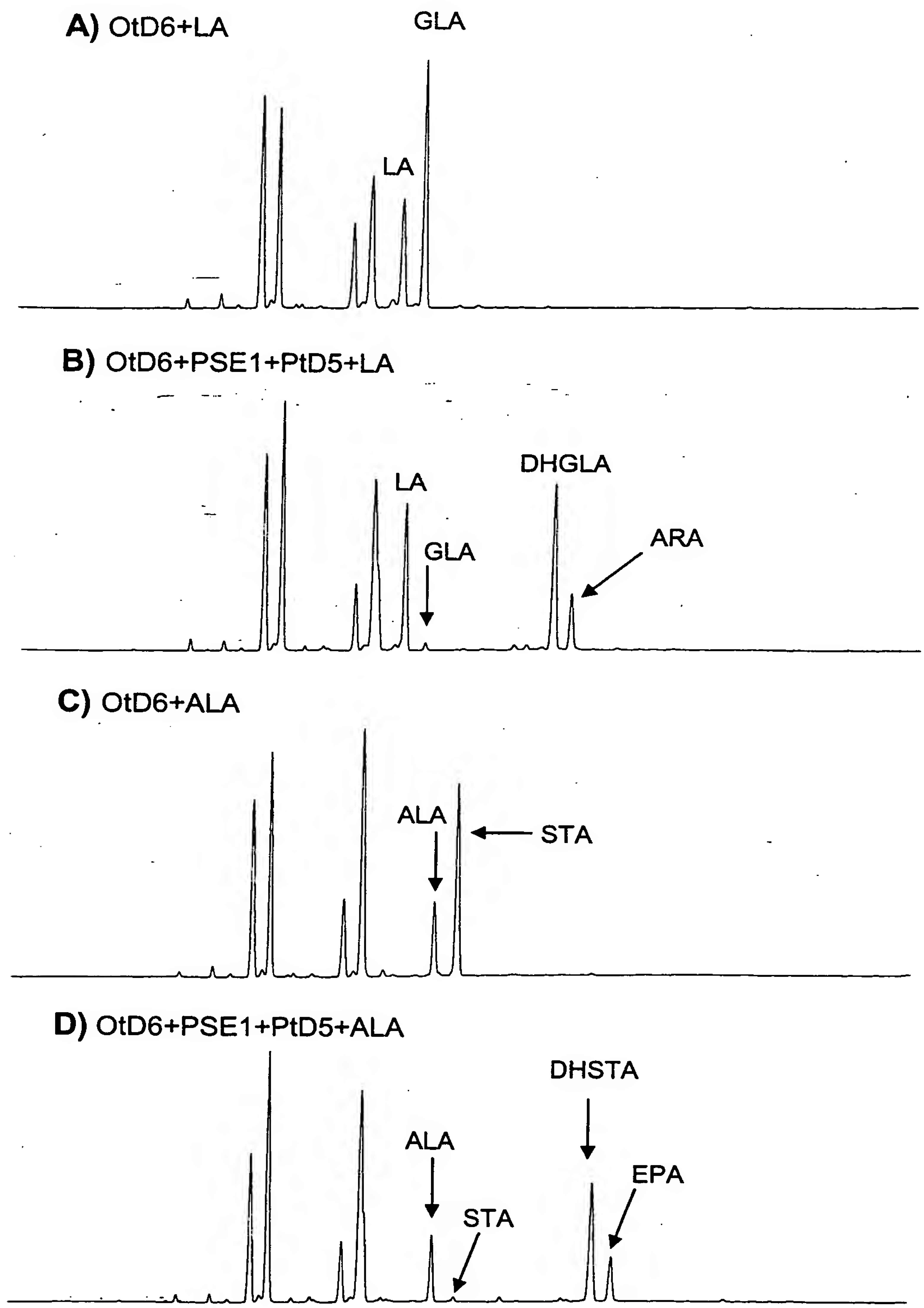


Figure 22: Expression of ELO(XI) in yeast

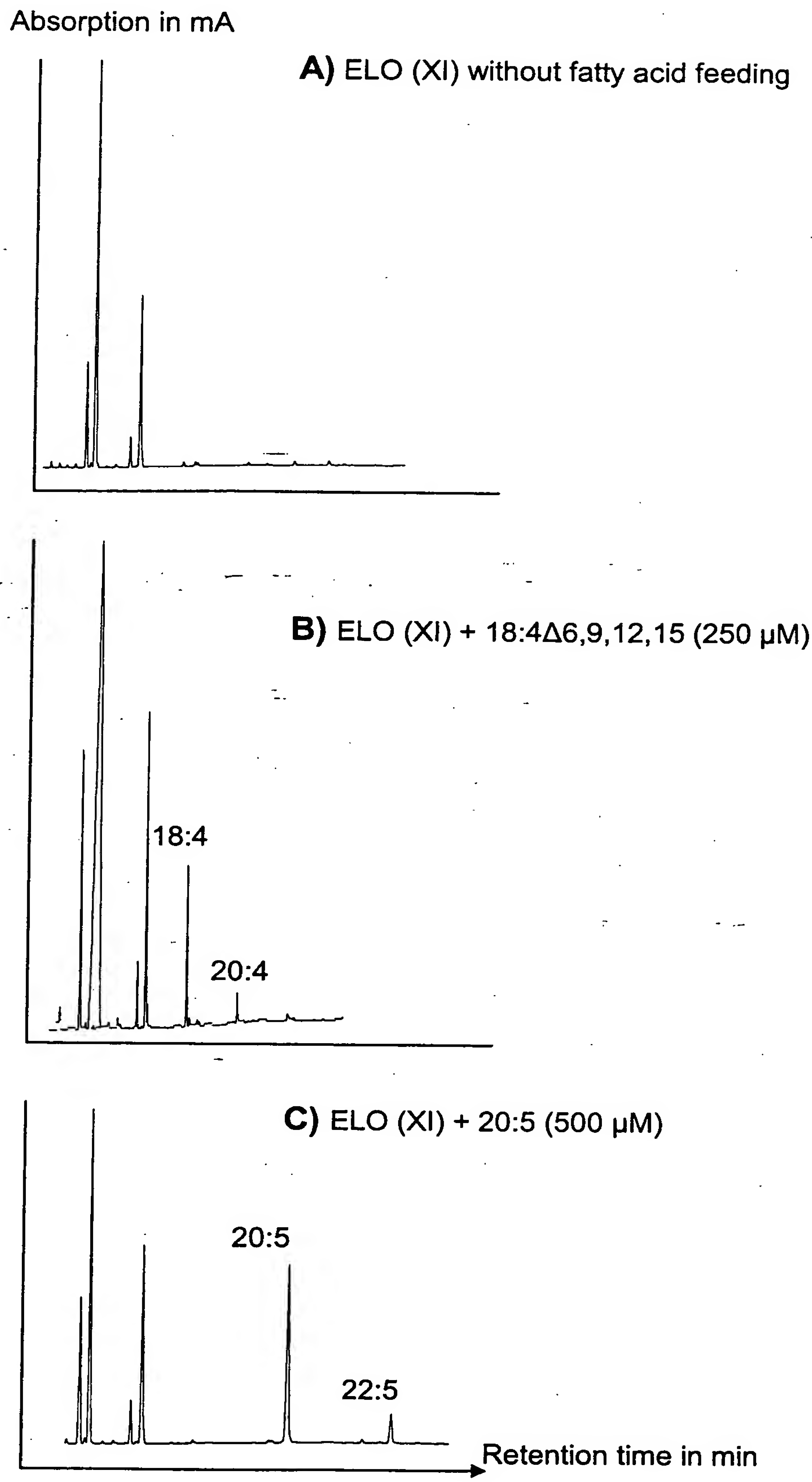


Figure 23:

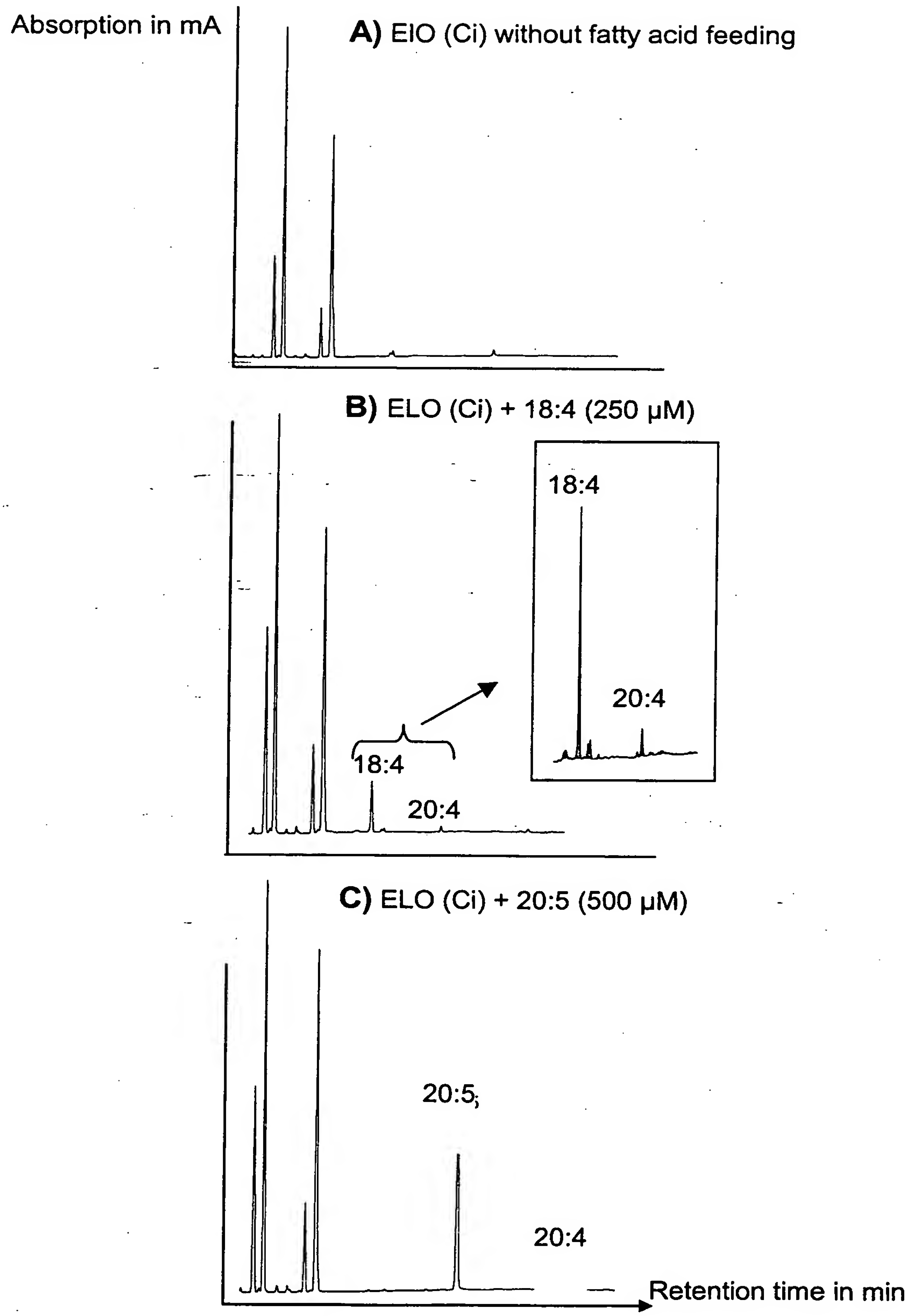
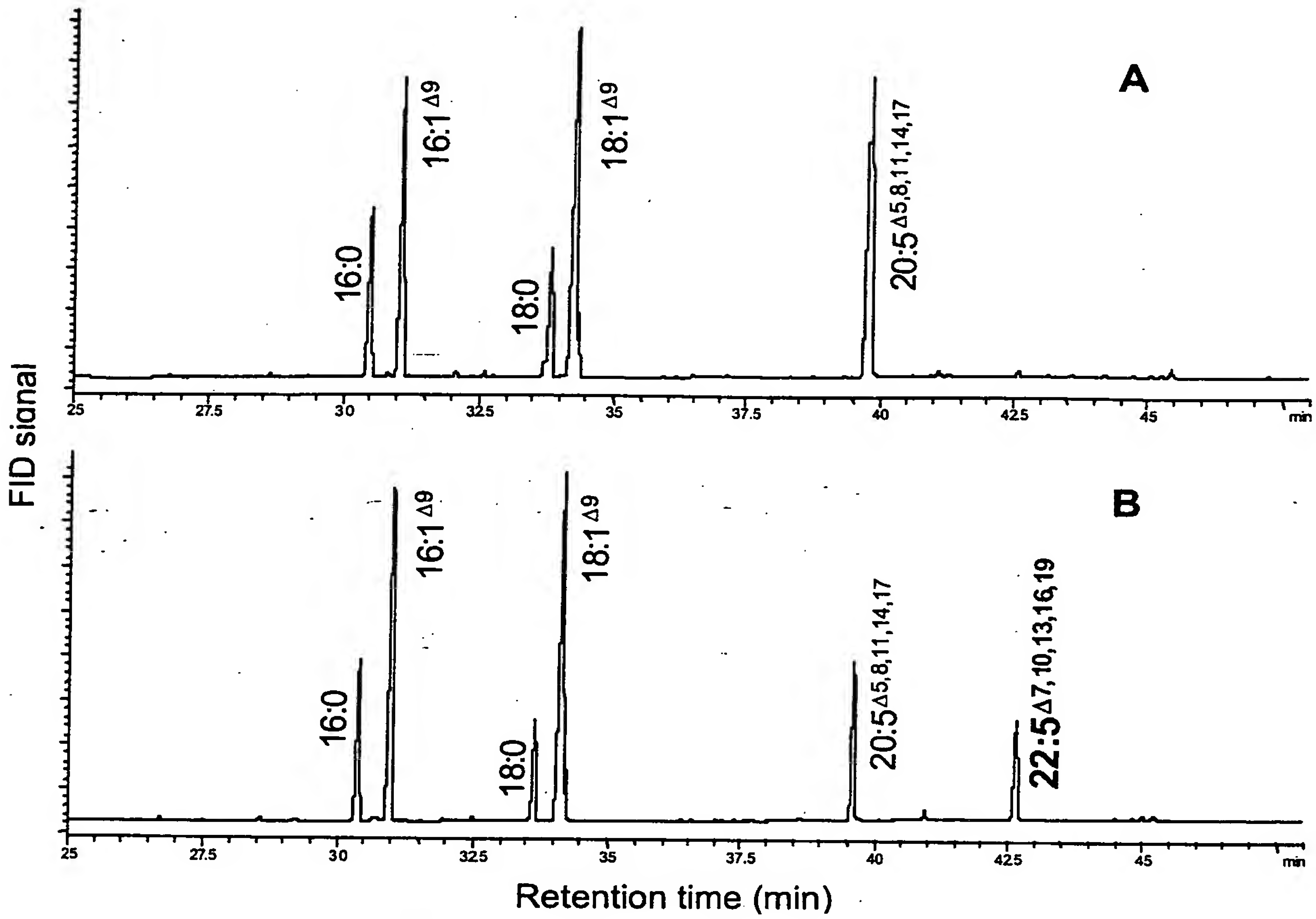

Absorption in mA
A) EIO (Ci) without fatty acid feeding
B) ELO (Ci) + 18:4 (250 µM)
18:4
20:4
18:4
20:4
C) ELO (Ci) + 20:5 (500 µM)
20:5;
20:4
Retention time in min

Figure 24: Elongation of eicosapentaenoic acid by OtElo1 (B) and OtElo1.2 (D), respectively. The controls (A, C) do not show the elongation product (22:5ω3).



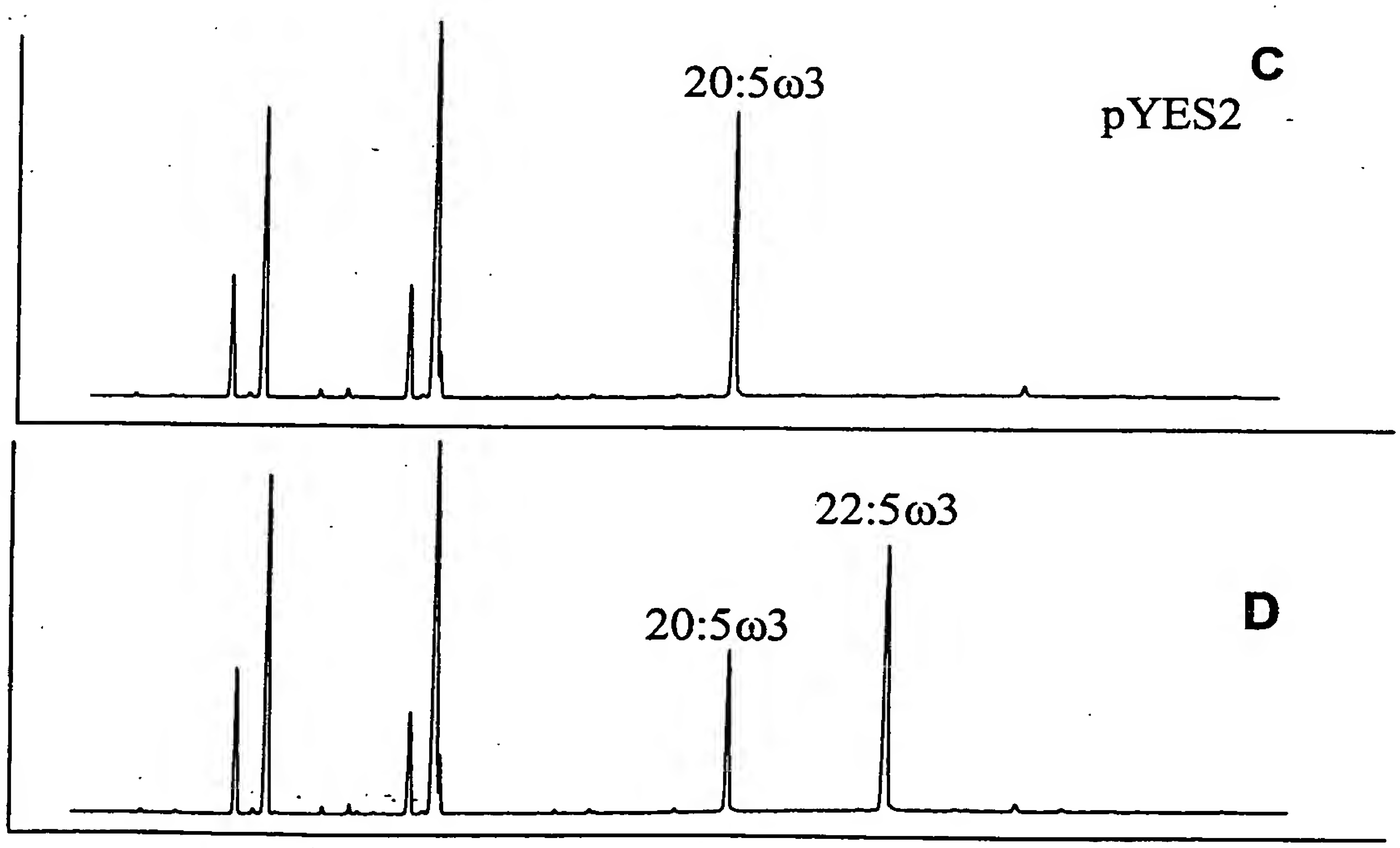



Figure 25: Elongation of arachidonic acid by OtElo1 (B) and OtElo1.2 (D), respectively. The controls (A, C) do not show the elongation product (22:4ω6).

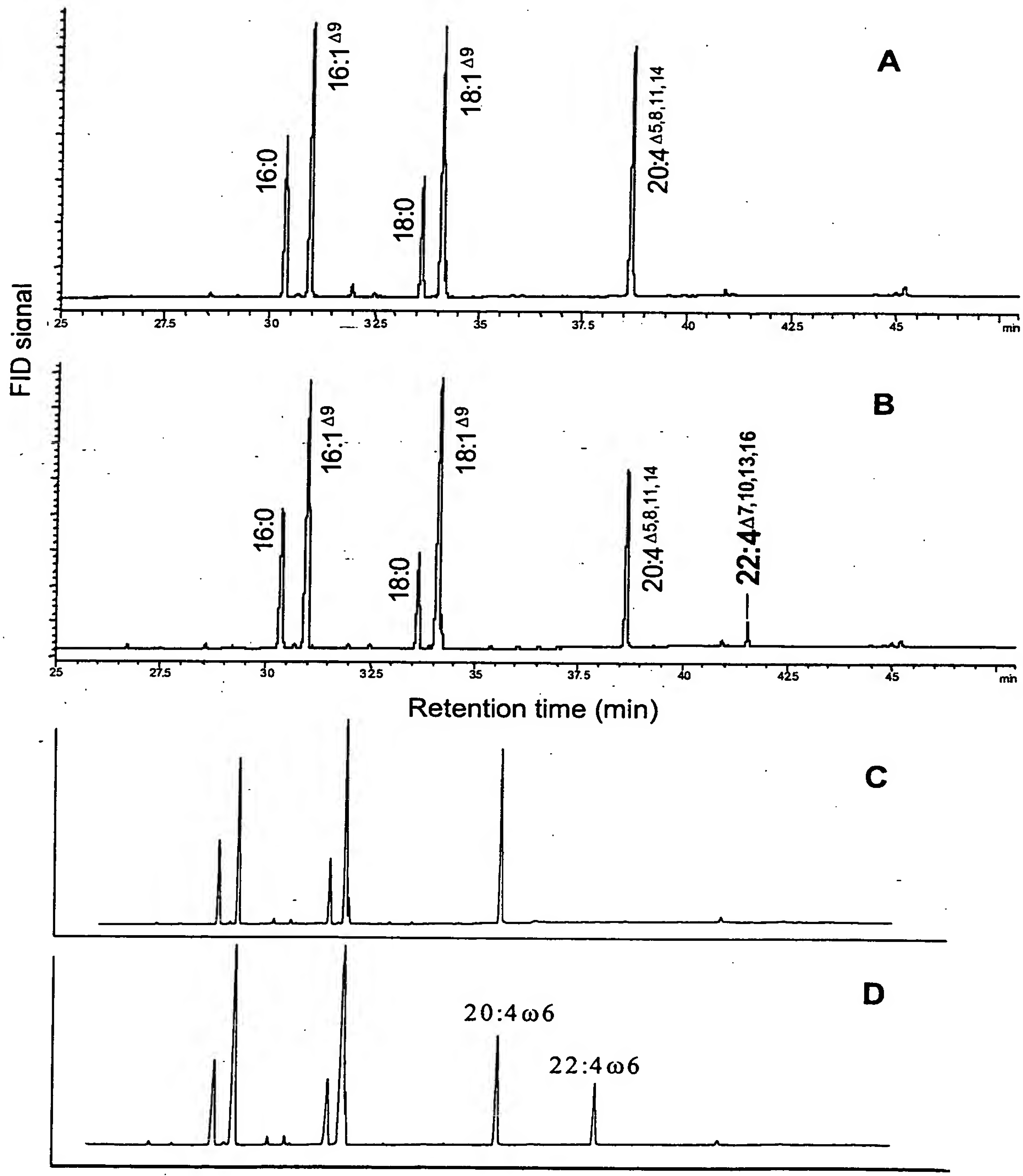


Figure 26: Elongation of 20:5n-3 by the elongases At3g06470.

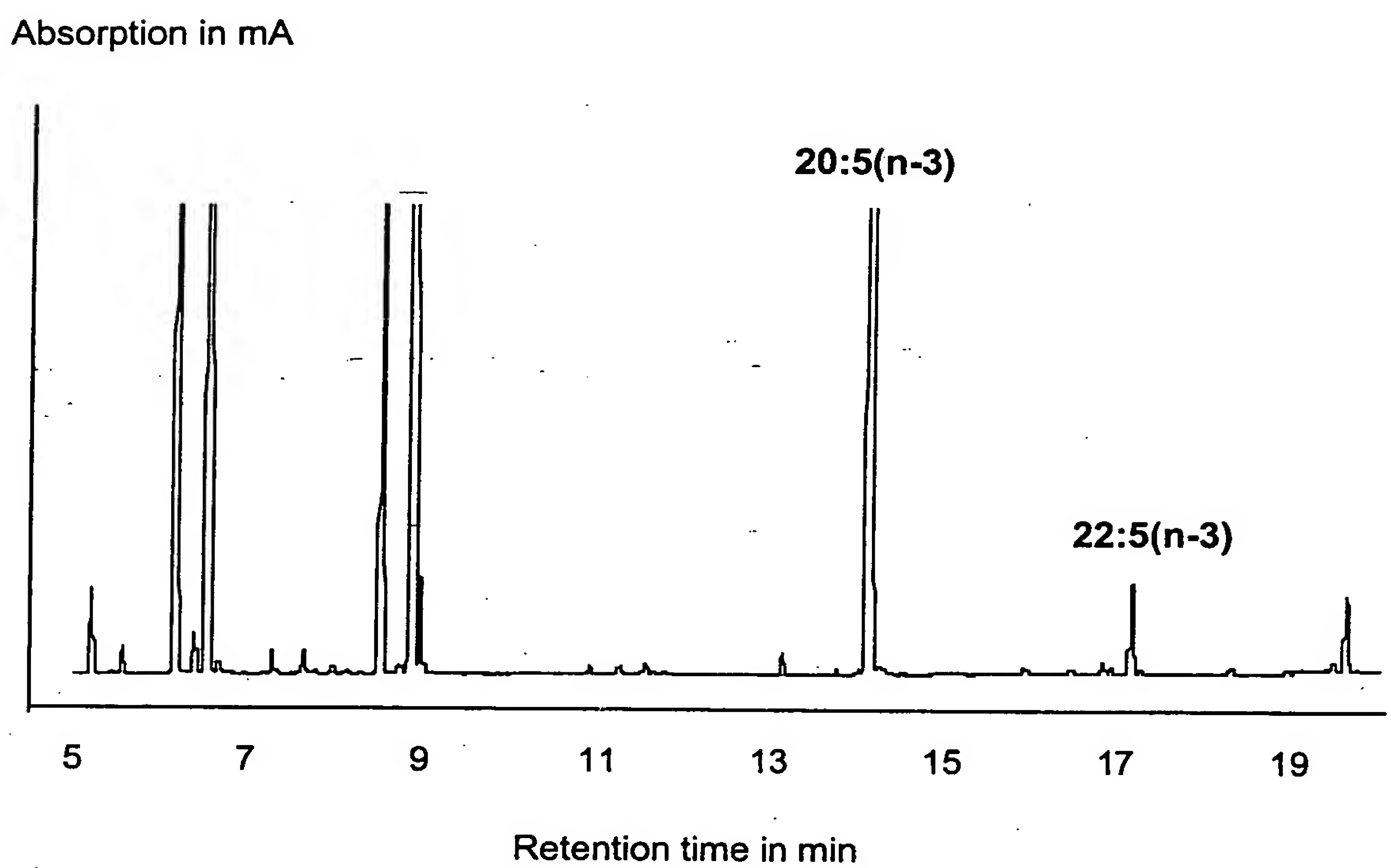


Figure 27: Substrate specificity of the Xenopus Elongase (A), the Ciona Elongase (B) and the Oncorhynchus Elongase (C)

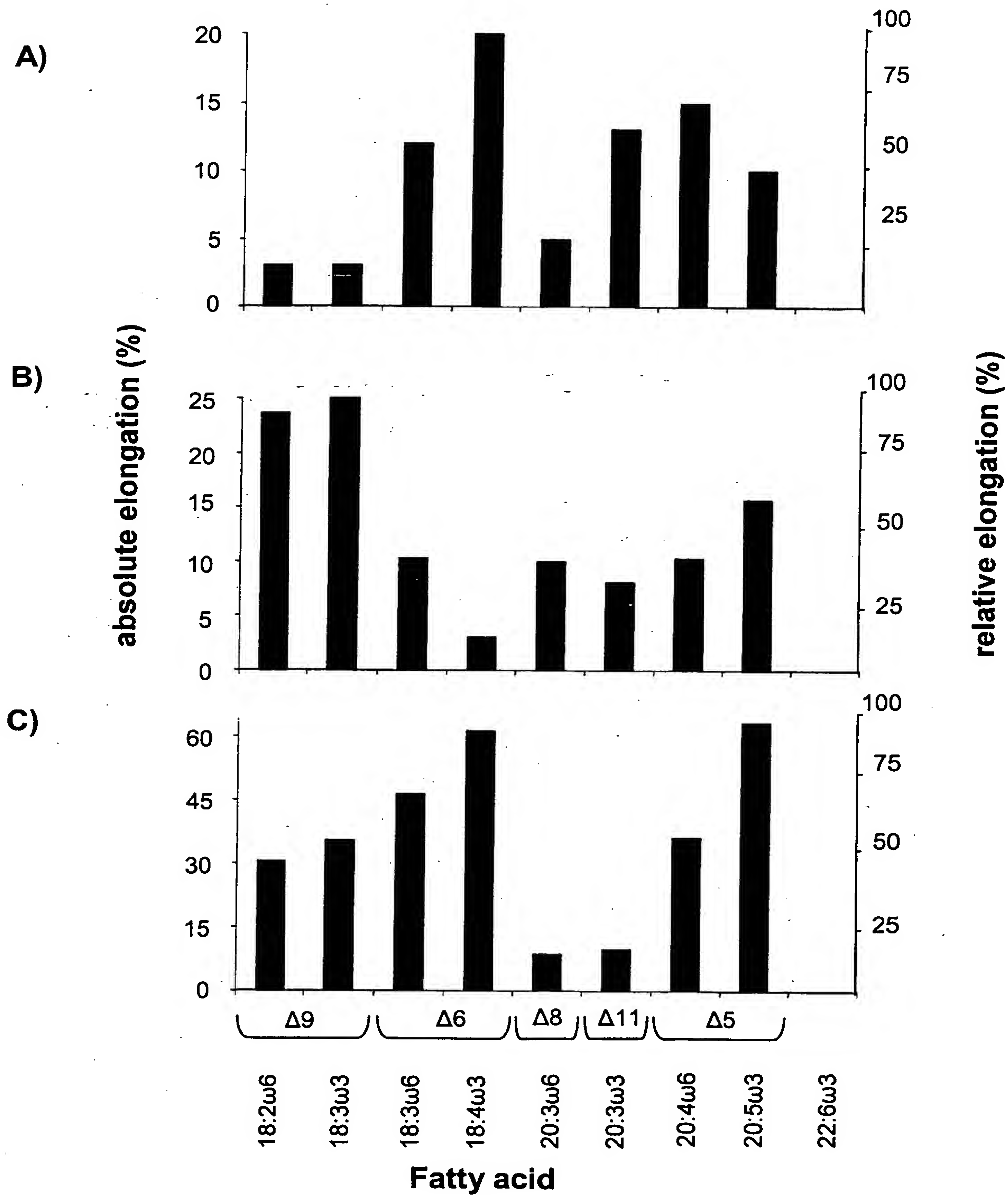


Figure 28: Substrate specificity of the Ostreococcus Δ5-elongase (A), the Ostreococcus Δ6-elongase (B), the Thalassiosira Δ5-elongase (C) and Thalassiosira Ostreococcus Δ6-elongase (D)

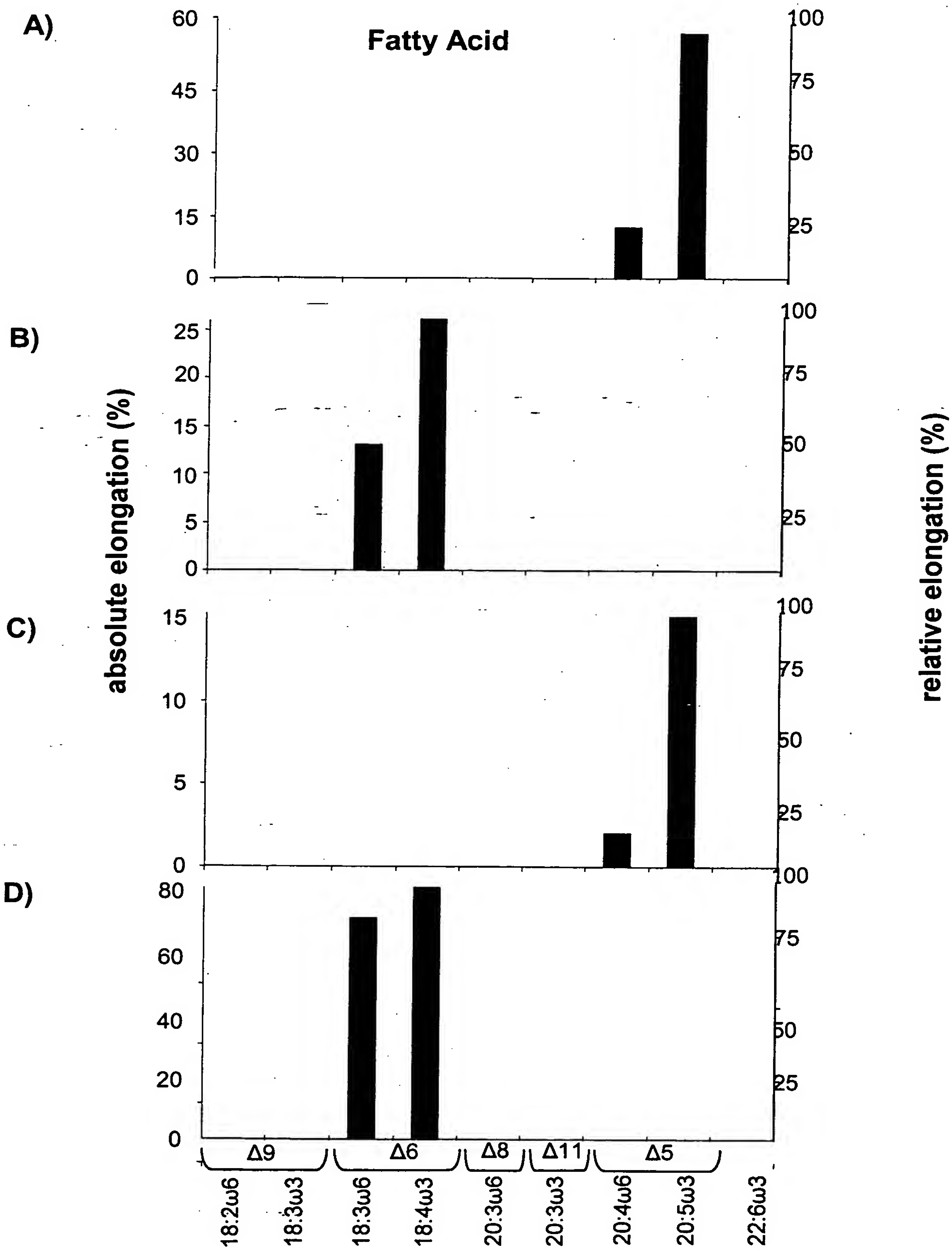


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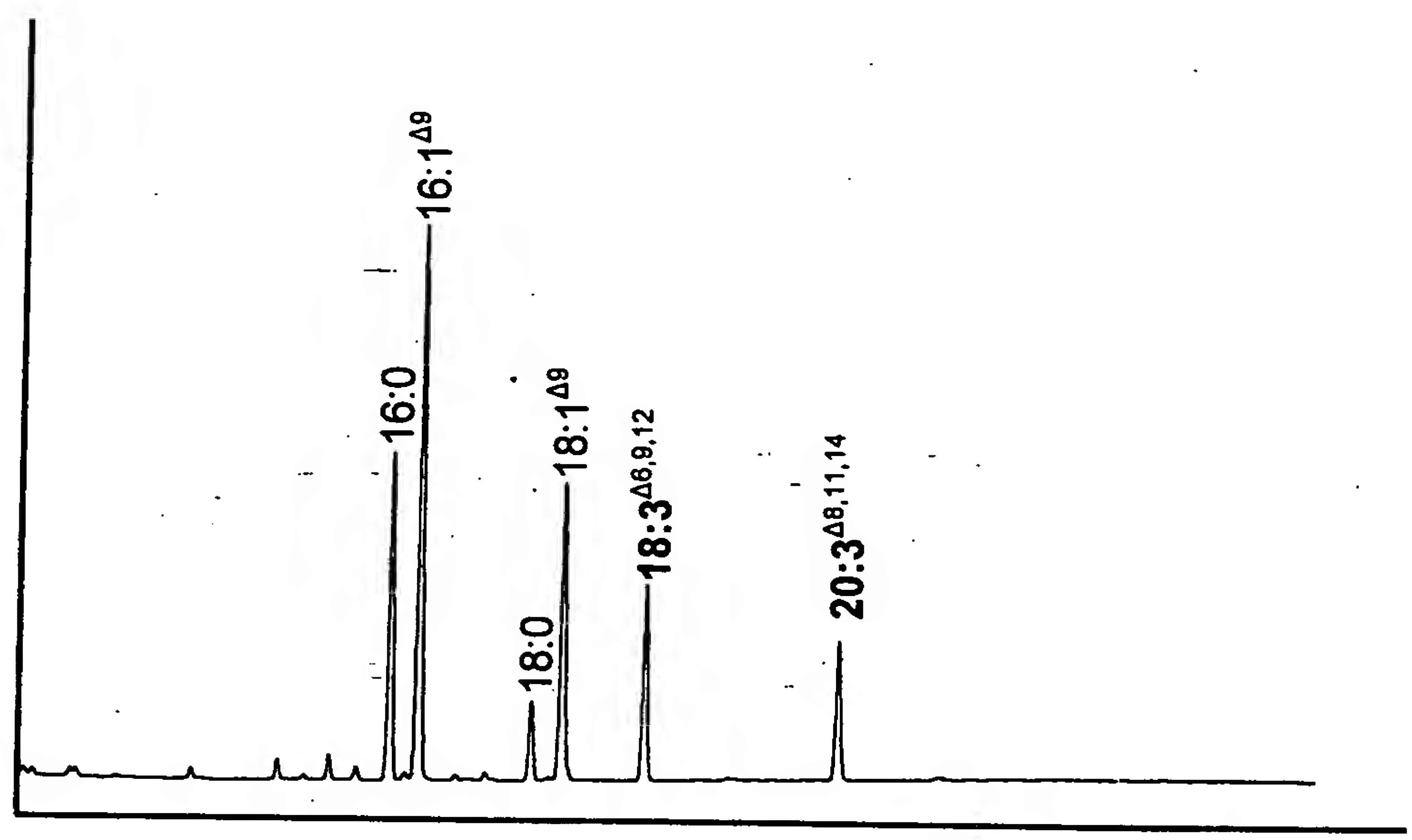
Figure 29: Expression of the Phaeodactylum tricornutum Δ6-elongase (PtELO6) in yeast. A) shows the elongation of the C18:$3^{\Delta6,9,12}$-fatty acid and B) the elongation of the C18:$4^{\Delta6,9,12,15}$-fatty acid

A)




B)

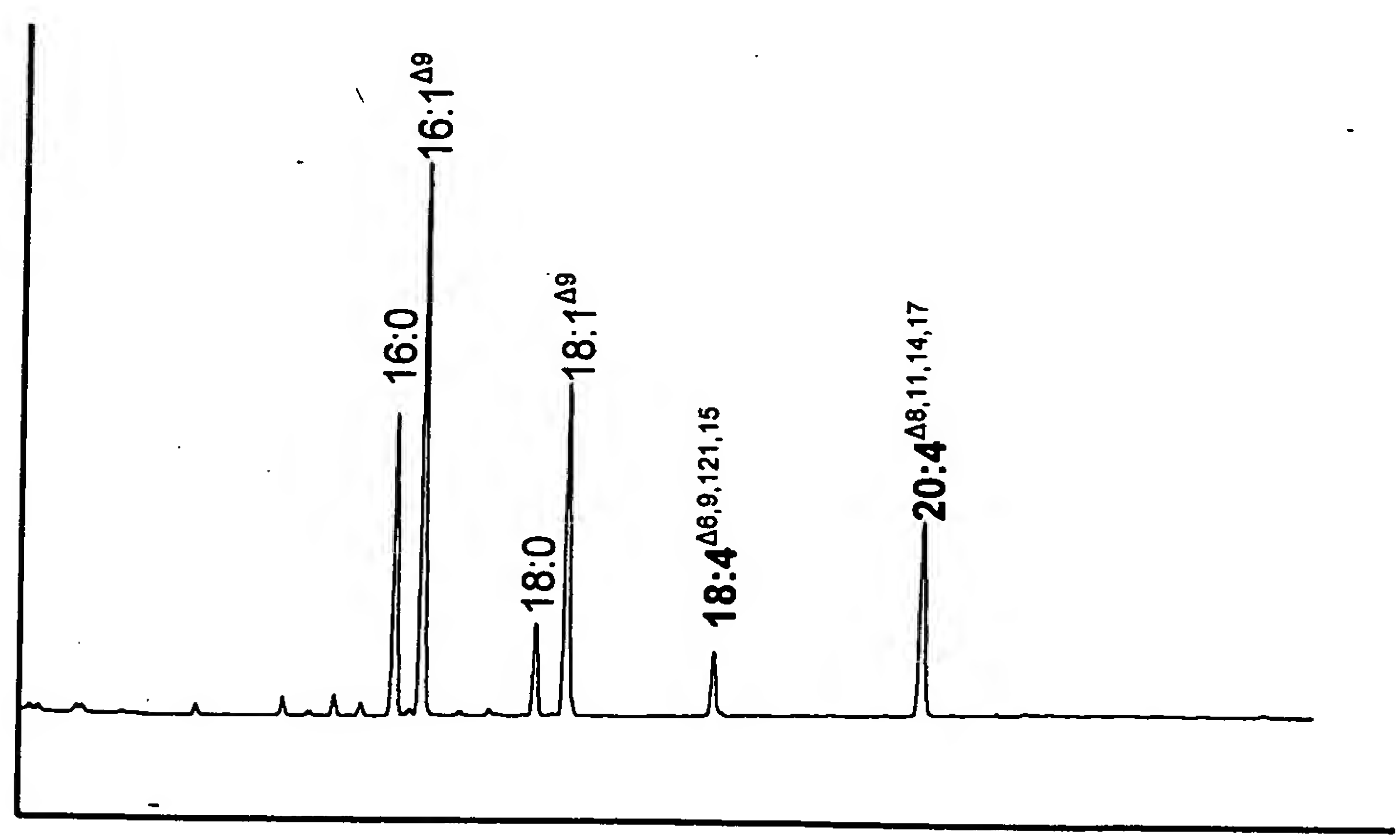


Figure 30: Figure 30 shows the substrate specificity of PtELO6 with regard to the substrates fed